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Tesis Doctoral 2017

Influencias ambientales en la expresión y selección de caracteres melánicos en el cernícalo vulgar (*Falco tinnunculus*)

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museonacionaldecienciasnaturales

UNIVERSIDAD AUTONOMA DE MADRID

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FACULTAD DE CIENCIAS

Departamento de Ecología

INFLUENCIAS AMBIENTALES EN LA EXPRESIÓN Y SELECCIÓN DE CARACTERES MELÁNICOS EN EL CERNÍCALO VULGAR (*FALCO TINNUNCULUS*)

Memoria presentada por David López Idiáquez para optar al Grado de Doctor en Ecología por la Universidad Autónoma de Madrid.

El doctorando

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"*Perseverance is a virtue of the less brilliant*"

-Santiago Ramón y Cajal

Ilustración y diseño de la portada: Lorenzo Pérez-Rodriguez

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RESUMEN

El mundo animal es una constante fuente de estructuras y comportamientos que por su naturaleza llamativa han atraído la atención de multitud de científicos. Estos rasgos de comportamiento cumplen un importante papel para los animales, ya que son sistemas de comunicación que determinan la resolución de multitud de conflictos, tanto entre individuos de la misma especie como entre individuos de diferentes especies. Las formas de comunicación animal son muy diversas y tienen lugar mediante señales, producidas por un emisor, y que a través del mensaje que transmiten, modulan la respuesta de los individuos receptores. En la comunicación entre individuos de la misma especie, la señalización puede tener lugar en diferentes contextos en función de los intereses de señalizadores y receptores. Uno de los escenarios es aquel en el que señalizadores y receptores tienen intereses comunes, ya que la eficacia biológica de una de las partes depende, al menos parcialmente, de la eficacia biológica de la otra. Este escenario tiene lugar, por ejemplo, cuando emisores y receptor están genéticamente emparentados, como sería el caso de la comunicación entre padres e hijos. Un contexto diferente es aquel en el que los intereses de emisor y receptores se enfrentan, como en los casos en los que los individuos compiten por recursos limitados. Finalmente, los intereses de emisores y receptores pueden divergir. Por ejemplo, durante la selección de pareja, los machos señalan su calidad para acceder al mayor número de parejas posible, mientras que las hembras usan estas señales para elegir a un único macho de la más alta calidad. Un requisito necesario para que estas señales sean efectivas y perduren evolutivamente es que las señales que se muestran sean honestas. La honestidad está basada en el coste diferencial que sufren individuos de baja o alta calidad al producir y/o mantener las señales. Desde un punto de vista evolutivo, la presencia de sistemas de comunicación estables es indicativa de que, al menos en promedio, las señales que se usan en ellos son honestas. Si no lo fueran, la selección natural favorecería a aquellos individuos que no reaccionan frente a las señales falsas y finalmente el sistema se desestabilizaría.

Es fácil darse cuenta de que todos los individuos de una misma especie no exhiben los mismos rasgos con igual intensidad, como se puede ver, por ejemplo, en la variabilidad del tamaño de las astas de los ciervos. La diferencia más evidente es que en esta especie los machos poseen astas mientras que las hembras carecen de ellas, además, el tamaño de las astas de los machos varía en función de diferentes características individuales. Este ejemplo nos sirve para ilustrar que la expresión de un rasgo está determinada por muchos factores que pueden ser inherentes a los individuos, como en el caso del sexo o la edad, o pueden estar asociados a la heterogeneidad ambiental, por ejemplo, a las fluctuaciones en los niveles de alimento. Además, estos factores no siempre actúan independientemente, pudiendo interactuar entre ellos. El estudio de los factores asociados a la expresión de las señales es de vital importancia para comprender tanto el mensaje transmitido como las presiones evolutivas que hay detrás de los rasgos y de los sistemas de comunicación donde se emplean.

El uso de rasgos o estructuras coloreados es una de las formas más habituales y conspicuas de emitir una señal. Las aves son uno de los grupos más coloridos dentro del mundo animal, exhibiendo rasgos de multitud de colores y formas. En las aves podemos distinguir dos tipos de coloración que en algunos casos pueden producirse simultáneamente. Por una parte, la coloración pigmentaria, que es consecuencia de la deposición de pigmentos como la melanina o los carotenos. Por la otra, la coloración estructural, en la que los colores se originan por la propia estructura microscópica de la pluma, como es el caso de los vistosos baberos iridiscentes de muchos colibríes.

Las coloraciones basadas en melanina son de las más comunes en el mundo animal. A partir de este pigmento se pueden generar muchos colores, sin embargo, las coloraciones melánicas más habituales son aquellas con colores negros, grises, marrones y marrones rojizos. La melanina se forma a través de un proceso llamado melanogénesis, en el que a partir del aminoácido tirosina se llega a producir tanto la eumelanina como la feomelanina, que son las dos formas principales en las que encontramos este pigmento y que dan lugar a las tonalidades negras y rojizas, respectivamente. Los rasgos basados en este pigmento cumplen un papel muy importante en la comunicación animal. Se ha mostrado, por ejemplo, cómo la presencia de un determinado rasgo melánico puede ser usada por las hembras a la hora de elegir pareja. Sin embargo, la función principal de las coloraciones melánicas es señalizar el estatus entre individuos de la misma especie, indicando la capacidad competitiva de sus portadores.

En esta tesis se describen diferentes mecanismos y funciones asociados a la expresión de estos rasgos melánicos, usando el cernícalo común (*Falco tinnunculus*) como especie de estudio. El cernícalo es una rapaz diurna de pequeño tamaño en la que los machos son aproximadamente un 20% menores en tamaño que las hembras. Además, también presentan dimorfismo cromático, siendo los machos los que exhiben un plumaje más vistoso. Concretamente, los machos tienen la cabeza, el obispillo y la cola de color gris mientras que el resto del plumaje es marrón con un número variable de motas. Las hembras presentan plumajes más apagados, mayoritariamente de color marrón salpicado con barras negras, a excepción de la cola y el obispillo que pueden presentar cantidades variables de coloración gris. Finalmente, la coloración de los juveniles es semejante a la de las hembras, exhibiendo también cantidades variables de gris en la cola y el obispillo. El cernícalo se distribuye más o menos homogéneamente por toda la Península Ibérica. Nuestra principal área de estudio es la región de Campo Azálvaro, entre las provincias de Segovia y Ávila. Esta área dispone de 62 cajas nido que han sido monitoreadas, marcando y siguiendo a todos los individuos desde el año 1994. Además, con objeto de incrementar el número de observaciones y la variabilidad ambiental en uno de los experimentos expuestos en esta tesis (Capítulo IV), durante un año también se estudió la población de cernícalos de Villalar de los Comuneros (Valladolid).

El punto central sobre el que pivotan todas las preguntas planteadas en esta tesis es estudiar los factores que modulan la expresión de diversos rasgos melánicos y su función en diferentes contextos sociales y ambientales. Estos aspectos se desarrollan de una forma

más específica a lo largo de 5 capítulos. Los dos primeros capítulos se centran en explorar los factores que modulan la expresión de los rasgos melánicos:

- En el **Capítulo II** se estudia cómo afectan la edad y la abundancia de alimento, en el número y el área de las motas presentes en el plumaje de machos y hembras adultas.
- En el **Capítulo III** se estudia el efecto de estos factores en la expresión de otro rasgo, la coloración del obispillo, únicamente en las hembras reproductoras ya que son las que presentan variabilidad en la expresión de la señal.

En los tres capítulos restantes se aborda la potencial función de los rasgos melánicos en diferentes fases del ciclo vital del cernícalo vulgar:

- En el **Capítulo IV** estudiamos el rol de la coloración del obispillo como una señal de estatus en un contexto de competición intrasexual (en hembras) durante las primeras fases de la reproducción.

- En el **Capítulo V** se explora la asociación ente la coloración del obispillo y la duración del periodo de dependencia de los volantones con las tasas de supervivencia en el primer invierno.

- En el **Capítulo VI** se estudia el rol de la coloración del plumaje y obispillo como indicadores de la personalidad durante el crecimiento de los pollos.

Finalmente, en el **Capítulo VII** se presentan y discuten de forma conjunta los resultados obtenidos en los capítulos anteriores.

ABSTRACT

The animal kingdom is a constant source of conspicuous structures and behaviours that have drawn the attention of the scientific community. These traits play a crucial role in animals' life, since they are the pillars where communication systems lay on and help individuals to resolve many conflicts, both within and between species. Animal communication can occur by very different means, but always in a bidirectional fashion that involves a sender, modulating the behaviour of the receiver. When the communication process occurs among individuals of a given species, signalling takes place in different contexts, depending on the interests of signallers and receivers. For example, signallers and receivers can share interests, as fitness benefits of one part may depend upon the fitness benefits of the other. This scenario can be found when individuals involved in the communication process are genetically related, like for instance, between parents and offspring. Alternatively, the interests of signallers and receivers can collide, when individuals compete for limited resources for example. Finally, the interests of signallers and receivers can diverge. For instance, during mate choice, males signal their quality aiming to obtain as many females as they can, while females may use these signals to find a single male of the highest quality. However, an essential requirement to obtain stable communication systems, is that the signals has to be a reliable proxy of individual condition. Signal honesty is based on the differential cost of production or maintenance paid by low and high quality individuals. Form an evolutionary perspective, the presence of stable communication systems is indicative that, at least in average, the signals used in them are reliable. If they were not reliable, Natural Selection will favour individuals that not react to those dishonest signals and, in the end, the communication system vanishes.

It is easy to realise that all individuals within a single species do not exhibit the same traits with equal intensity, as can be seen for example, in the antlers of red deer. The most obvious difference is that in this species, males and not females show conspicuous antlers. In addition, among males there is also a variance in the size of their antlers depending on different individual characteristics. This example perfectly illustrates that the expression of a certain trait, secondary sexual in this case, is determined by several factors, that can be inherent to the individual, like sex or age, or be associated with environmental heterogeneity, like food availability. Besides, these factors do not act in isolation, and they can interact between them. The study of the factors that modulate signal expression is crucial to understand both, the message that is transmitted and the evolutionary pressures behind the expression of the trait.

The use of colour-based traits is one of the most typical ways of signalling. Among terrestrial animals, birds are of the most coloured groups, exhibiting traits varying in multiple colours and shapes. These colourations can be grouped in two different categories. First, pigmentary colouration, whose physiological origin occurs by the

deposition of pigments like melanin or carotenoids. Second, structural colouration, in which colours are generated by the interaction of the microscopic structure of the feather and light, as for example the iridescent gorgets present in many hummingbirds.
Melanin-based traits are one of the most common in the animal kingdom. This pigment is responsible of many colours, however, melanins are, usually, associated with colours like black, grey, brown and reddish-brown. Melanins are generated from the amino acid tyrosine, in a process known as melanogenesis. In this process, two different types of melanin can be produced, eu-melanin and pheo-melanin, that are responsible of blackish and brownish colorations respectively. Melanin-based traits have a crucial role within animal communication. It has been, for example, shown that the presence of certain melanin-based traits can be used by females to choose their mates during mate choice, reflect individual age or reliably show individual quality to name some. Still, the main role of these traits has been pointed towards a signalling function of the individual status, showing the competitive capabilities of the bearers.

In this thesis, I describe the different mechanisms and functions associated with the expression melanin-based traits, using the common kestrel (*Falco tinnunculus*) as study species. Kestrels are medium-sized diurnal raptors, exhibiting an reversed sexual dimorphism, where males are 20% smaller than females. In addition, they also are dimorphic in coloration, males being more conspicuous than females. Specifically, males have grey heads, rump and tails, and brown bodies with a variable number of black spots covering it. Females exhibit duller plumages, mostly brown with black bars in the body, except in the rump and tail, where they can present a variable proportion of grey colouration. Kestrels have a homogeneous distribution in the Iberian Peninsula, and our study areas are located in central Spain. First, Campo Azálvaro region, located between Segovia and Ávila, there are 62 nest-boxes that have been subjected to an individual-based monitoring since 1994. Second, with the aim of increasing the number of observations and the environmental heterogeneity, we also studied a kestrel population located in Villalar de los Comuneros (Valladolid) during the experiment performed in Chapter IV.

The pivotal point of this thesis is to study the factors that modulate the expression of different melanin-based traits and their function in different environments. These aspects are developed in 5 different chapters, in addition to a general introduction (Chapter I). The first two are focused on exploring the factors that modulate the expression of melanin-based traits:

- In **Chapter II**, I explore how age and environment modulate the expression of the number and size of the spots shown in the plumages of adult males and females.

- In **Chapter III**, I study the influence of the above-mentioned factors in a different trait, rump colouration, only in adult females, as is the sex where there is variation in the expression of this trait.

In the remaining three chapters, we explored the function that melanin-based traits have during different stages of kestrels' life-cycles.

- **Chapter IV**, I focus on the role of female rump colouration as a signal of status within an intra-sexual competition context during the pre-laying period.
- In **Chapter V**, I explore the association between the duration of the post-fledgling dependence period, offspring rump colouration and survival rates to the first winter. In addition, I study the influence that this period has on future reproduction of parents.
- In **Chapter VI**, I explore the role of plumage and rump colouration as personality index during the nestling growth.

Finally, in **Chapter VII**, I present and discuss in an integrative manner the main results obtained in this thesis.

Chapter I

INTRODUCTION

1. ANIMAL COMMUNICATION

The animal kingdom is a constant source of amazing structures and behaviours that have drawn the attention of society in general, and biologist in particular, for a long time. From the classical train present in male peacocks (*Pavo cristatus*; Petrie, Tim & Carolyn 1991) to the recently discovered huge and complex geometric structures made in the seabed by the small male pufferfish (Family: *Tetraodontidae*, *Torquigener sp.*; Kawase, Okata & Ito 2013), the diversity of animal signals is astonishing. It was in 1872, with the publication of *The Expressions and Emotions in Man and Animals* by Charles Darwin, when the foundations for the comparative study of the animal signalling were laid (Laidre & Johnstone 2013). Darwin argued that many signals have a universal basis, working under the same principles in different species, and suggested that their role was to convey information about the signallers motivational or emotional state (Darwin 1872). However, it was not until the empirical work developed by the Nobel Prize winners (1973), Konrad Lorenz, Karl von Frisch, and Nikolaas Tinbergen when the proper study of animal signalling and communication started, and suggested the bases for the field of animal behaviour.

According to Edward O. Wilson, animal communication occurs when the action or cue given by an individual is perceived by a receiver. Thus, effective communication exists when such interchange of information alters the behaviour of another organism in an adaptive fashion to either one or both of the participants (Wilson 1975). An effective communication is a crucial feature in the life of all animals, as it can determine a range of intra- and inter-specific interactions that, in the end, can modulate their fitness. Communication takes place using signals, some of which can be incredibly conspicuous like those shown by, for example, birds-of-paradise (Class: *Aves*, Family: *Paradisaeidae*; Laman & Scholes 2012). Others, although they are rather subtle, like the electrical signals used in many fish (Lissmann 1958), the ultrasonic cries of bats (Griffin 1958) or the ultraviolet reflectance present in plants and animals (Sheldon *et al.* 1999), are equally functional. Signals can be defined as traits that are produced by senders, which transmit information through the environment and which help receivers to decide whether and how to respond to senders (Irschick, Briffa & Podos 2015). Signalling takes place in different contexts depending on the interests of both signallers and receivers, and the selective pressures acting on those contexts might be rather different (Searcy & Nowicki 2005). The first signalling scenario occurs when the interests of the signaller and receiver overlap, when the fitness benefits of one depends, at least partially, on the fitness of the

other (Searcy & Nowicki 2005). This usually happens whenever two individuals are genetically related. For instance, the communication process between parents and offspring, where the interests of signallers (offspring) and receivers (parents) can converge, at least during some part of their lifecycle, as they share many of their genes. Alternatively, signals can be used in a context where the interest of the individuals diverge, as for example during mate choice. As a general trend, due to a divergent investment in reproduction, the interests of males and females are not the same and emerge an inter-sexual conflict. Such diverging interest lays on the idea that males signal their quality in order to obtain as many mates as they can, to maximize the number of offspring produced. In turn, females use those signals to select a mate with the primest quality and thus maximize the viability of the offspring raised. Finally, signalling can occur when the interests of the involved individuals oppose, as commonly happens between individuals of the same species and sex when competing for limited resources like food or breeding sites (Searcy & Nowicki 2005).

Regardless of the context in which animal communication takes place, a key question within the field of animal behaviour arises: what keeps individuals away from cheating? By cheating, an individual shows-off and advertises qualities that do not accurately reflect their own. As communication is a major determinant of individual fitness, one could expect that dishonest signalling may increase more effectively either survival prospects or reproductive output, or both. However, Natural Selection does not favour cheating or cheaters, since the expression of those signals does not seem to confer any fitness benefits to individuals mated with cheaters, as cheaters will be easily spotted and out-competed by rivals. Thus, cheating is not an evolutionary stable strategy, since there are no fitness benefits for cheaters and such strategy will not persist in the population. Therefore, it would be expected that the information contained in the signals is honest to the bearer, rising an even more interesting question: which are the mechanisms behind the honesty of animal signals?

2. SIGNAL HONESTY AND THE HANDICAP PRINCIPLE

Different hypotheses have been proposed to explain signal honesty and the mechanisms underlying it. It is interesting to point out that signals can be honest *per se*, without any mechanism ensuring their honesty. This absence of cheating can be achieved by different mechanisms. For example, the anatomical or physiological constraints involved in signal expression can determine that only individuals in prime condition exhibit enhanced versions of the traits ("constraints hypothesis"; Maynard Smith & Harper 2003; Searcy & Nowicki 2005), like, for instance, the roars produced by males in the red deer (*Cervus elaphus*; Clutton-Brock & Albon 1979). Another mechanism that has been proposed to avoid cheating is the individually directed scepticism hypothesis (Silk, Kaldor & Boyd 2000). According to this idea, honesty relies on the successive encounters of certain individuals, in which receivers remember the reliability of past signals and adjust their current trust and response to signallers accordingly. In this context, the benefits of being

dishonest in a first encounter can be outweighed by obtaining lower benefits in future interactions with that given signaller (Silk *et al.* 2000; Searcy & Nowicki 2005). However, the main evolutionary mechanism behind the idea of free cost honest signalling was proposed in 1930 by Ronald A. Fisher to explain why some males exhibit extravagant ornaments within a Sexual Selection context, a hypothesis called "the run-away" (Fisher 1930). According to Fisher, the presence of these traits begins by an arbitrary preference of the choosy sex for a specific trait, that does not necessarily provide information about the bearer, or has any functionality. This preference confers a fitness advantage to the individuals bearing the trait over others in the population, passing on its genes to the next generation, resulting in a positive feedback loop where the trait becomes more exaggerated in each generation (Fisher 1930). After several generations, the relative fitness benefit of expressing a trait is reduced because the genes are spread out in the population, and therefore the benefits of bearing an enhanced trait are overwhelmed by the costs generated by Natural Selection. After such first loop, the process starts over when, again by chance, other trait is positively selected and gain fitness benefits. This repeated process represents a run-away or an evolutionary escape to express a sexual signal to another. In this context, since the importance of the signal lies on its own expression rather than in the information that it could convey, there is no place for cheating.

Although the abovementioned ideas have received empirical support (Fisher 1930; Bergstrom & Lachmann 1998; Silk *et al.* 2000; Maynard Smith & Harper 2003; Searcy & Nowicki 2005), the main hypothesis that has tried to explain the evolution and maintenance of an evolutionary stable system of animal signals is the handicap principle, proposed by Amotz Zahavi in 1975. Zahavi defended that, in multiple communication contexts, like those between parents and offspring, predators and preys, or within Sexual Selection, honesty is associated with the production and/or maintenance costs of the signals (Zahavi 1975). According to Zahavi, only prime quality individuals can afford the production and/or maintenance of the most elaborate signals without experiencing survival costs (Zahavi 1975). The ideas originally proposed by the handicap principle were received with scepticism (Maynard Smith 1976; Bell 1978; Grose 2011), particularly for those that thought that the handicap itself can be easy and cheap to cheat (Dawkins 1976). However, in 1990 and focusing on secondary sexual traits, Alan Grafen provided a mathematical background for Zahavi's verbal ideas (Grafen 1990). Grafen showed that the handicap principle is theoretically plausible when the costs of expressing a signal diverge for high- and low-quality individuals (Fig.1.1; Grafen 1990). As such, only high-quality individuals are able to stand the enhanced costs of producing or maintaining the most elaborate signals. Beyond Grafen's theoretical models within a Sexual Selection context, the handicap principle can theoretically work also during agonistic encounters (Enquist 1985) or in competitive contexts for resource acquisition (Maynard Smith 1991; Johnstone & Grafen 1992). Still, the empirical evidence showing that high- and low-quality individuals pay a differential cost for signalling is scarce (Johnstone 1997; Kotiaho 2001). Two seminal papers published by Anders P. Møller and Florentino de Lope in 1994 and by Janne S. Kotiaho in 2000 have been able to

experimentally show these differential signalling costs. First, Møller and de Lope modified the length of the barn swallows (*Hirundo rustica*) tail and explored its effects on survival. They found that survival rates were higher for individuals with reduced tails, evidencing the cost of survival associated with the expression of such a sexual trait. In addition, they also found that naturally long tailed individuals had increased probabilities of surviving to the next breeding season in relation to experimentally elongated tails. As in this species the length of the tail is a proxy of individual quality, this result suggests that the cost of producing or maintaining longer tails is paid in terms of survival (Møller & de Lope 1994). A second study demonstrating these differential costs was performed by Kotiaho using wolf spiders (*Hygrolycosa rubrofasciata*) as study species (Kotiaho 2000). In this spider, drumming rate is a secondary sexual trait and drumming males had reduced survival rates when compared with non-drumming males (Mappes *et al.* 1996; Kotiaho *et al.* 1996). Kotiaho, by modifying both the individual condition and the drumming rate of males, showed that there were differential survival rates depending on their condition, with drumming-males in higher condition having enhanced survival rates than drumming-males in low condition (Kotiaho 2000).

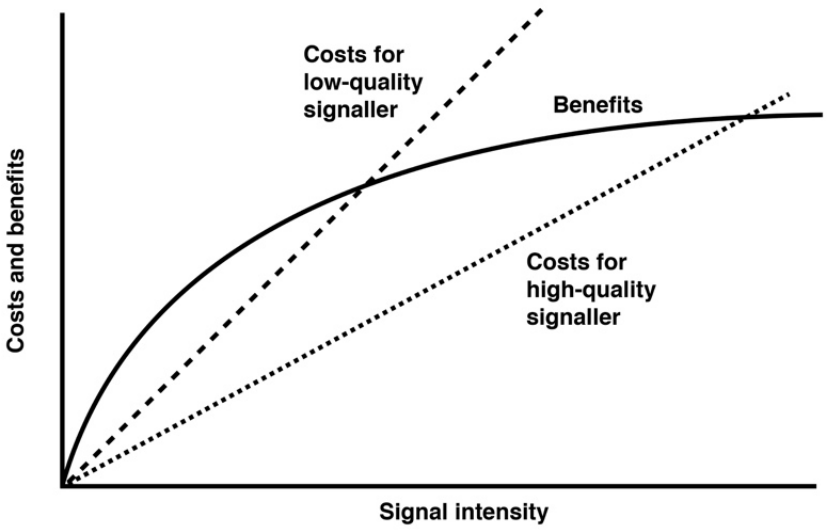

Figure 1.1: Illustration of the way in which differential costs can maintain honesty. For the expression of a signal of the same intensity, high-quality individuals (pointed-line) pay a lower cost than low quality ones (dashed-line). Therefore, for the same amount of energy invested, high-quality individuals are able to express enhanced signals and thus obtain increased benefits. Adapted from Johnstone (1997).

Above, I have shown the main theories that explain signal honesty and the presence of evolutionary stable signalling systems. Nowadays, the idea proposed by the handicap principle, stating that signal honesty is tightly associated with the production or maintenance costs, is the most accepted to explain the evolution of stable signalling systems. Therefore, the concept of cost is the pivotal idea behind the evolutionary ecology of animal behaviour. Still, it is interesting to bear in mind that the costs associated with signal production or maintenance are not fixed and can be modulated by different factors,

both intrinsic and extrinsic to the signallers. Thus, disentangling the role of the factors that mediate the cost of producing or maintaining a signal is of great relevance in order to fully comprehend the evolution of animal communication.

3. FACTORS INFLUENCING THE EXPRESSION OF ANIMAL SIGNALS

As a matter of fact, all individuals within populations are not identical, showing in all species some degree of between-individual variability in different features, for instance in their signal expression (Dale 2006). Due to their role in communication, signalling traits are particularly sensitive to this between-individual variation (Dale 2006). Several examples, like the diversity of nuptial plumages of male ruffs (*Philomachus pugnax*; Hogan-Warburg 2002) or the variability of the roars in red deer (Clutton-Brock & Albon 1979), evidence this intra-specific variability in signal expression. This divergence in the expression of signalling traits arises through the mediating influence of different factors, some inherent to the individual, like their sex or age, and some associated with the environment they live in, like food availability or competition with other individuals. In addition, the forces modulating the expression of the signals are not independent and can interact between them. Below, I discuss the role of the factors modulating the expression of the traits studied in this thesis and the potential interactions between them.

(a) Individual characteristics

Sex

In many species, males and females face different selection pressures that lead to a divergent expression of different signalling traits (sexual dimorphism; Heinsohn 2005). The context where this divergence is more evident is on the expression of secondary sexual traits (SST), defined as the traits that confer a reproductive advantage, either for increasing mate opportunities or producing more offspring, or both. One of the most paradigmatic examples of sexual dimorphism in the animal kingdom is the peacock, where males display flashy eye-spotted feathers in their train during courtship (Petrie *et al.* 1991). The expression of these extravagant traits is not exclusive of birds and they are also present in other taxa, like the silver-backs in male gorillas (*Gorilla gorilla gorilla;* Margulis, Whitham & Ogorzalek 2003), the differences in colouration within the fishes of the genus *Etheostoma* (Mendelson 2003), the horns exhibited by many beetle males in the sub-family *Dynastinae* (Family: *Scarabaeidae*; Emlen *et al.* 2012), or the different colouration shown by male side-blotched lizards (*Uta stansburiana*; Sinervo & Lively 1996). Despite males are usually more ornamented than females, this is not always the case, since females can be the most conspicuous sex in species like the red-necked phalaropes (*Pharalopus lobatus*; Reynolds 1985) or the jacanas (*Jacana jacana*; Emlen & Wrege 2004). Regardless of which sex shows the most conspicuous sexual traits, it is

common that the expression of SST can be counter-adaptive and, rather than enhancing individuals' fitness benefits, they seem to do exactly the opposite (Darwin 1871). This apparent paradox is caused because these traits are generated by Sexual Selection rather than by Natural Selection (Darwin 1859; 1871). This idea was originally suggested by Charles Darwin, in *On the Origin of Species by Means of Natural Selection* (1859), who fully developed it later on as a topic on its own, in *The Descent of the Man and Selection in Relation to Sex* in 1871. Darwin stated that, the expression of these secondary sexual traits leads to increased reproductive benefits (Darwin 1871). Thus, the individuals exhibiting enhanced SST will be able to transmit their genetic heritage more effectively than those bearing duller or less conspicuous versions of the traits, being in that sense an evolutionary force as strong as Natural Selection (Andersson 1994). Sexual Selection theory remained dormant from Darwin's time until the 1960s, with the notable exception of the works by Ronald A. Fisher and Julian S. Huxley in the 1930s (Fisher 1930; Huxley 1938), but today is one of the fundamental topics in Evolutionary Ecology (Krebs & Davies 1991). According to Darwin, Sexual Selection can occur in two different contexts (Darwin 1871). First, it can work within an intra-sexual scenario, where Sexual Selection leads to the presence of traits that enhance the abilities to compete against individuals of the same sex in order to gain access to potential mates. Second, it can work within an inter-sexual context, where Sexual Selection favours the presence of traits that are preferentially selected by the opposed sex (Darwin 1871; Andersson 1994). Two different ideas have been proposed to explain the evolution of this "preference" towards elaborated SST (Kirkpatrick & Ryan 1991). Direct (non-genetic) benefits models state that the preference towards more ornamented individuals is based on the benefits obtained by the choosy sex to itself, like nuptial gifts, increased parental care or access to limited breeding sites (Trivers 1972). Alternatively, models based on indirect (genetic) benefits state that, by mating with more ornamented individuals, females will benefit by acquiring "good genes" for their offspring (Kirkpatrick & Ryan 1991; Andersson 1994; Kokko *et al.* 2002; 2003). On the one hand, these genes may have no other function beyond generating the chosen trait, as proposed by the Fisherian run-away, where there is an arbitrary preference for some trait (Fisher 1930). On the other hand, these genes can confer different benefits, like parasite resistance (Hamilton & Zuk 1982), the ability to carry a "handicap" (Zahavi 1975) or being more compatible with a potential partner (non-additive genetic benefits; Trivers 1972).

The study of Sexual Selection has been mainly biased towards males, although females can benefit from exhibiting conspicuous traits within a Sexual Selection context (Amundsen 2000; Amundsen & Pärn 2006; LeBas 2006), since the presence of these traits will allow mates or competitors to assess female quality (Kraaijeveld *et al.* 2004; Amundsen & Pärn 2006). This has been shown, for example, in bluethroats (*Luscina svecica*) and in rock sparrows (*Petronia petronia*) where females displaying enhanced ornaments are preferentially selected by males (Amundsen, Forsgren & Hansen 1997; Griggio *et al.* 2005). In addition, the expression of these traits has been shown to be condition-dependent and costly to produce. Still, due to the different selection pressures faced by males and females, the expression of female ornaments is not as straightforward

as it is in males. The fact that, in general, females are the main responsible sex of reproductive investment can lead to a well described trade-off between ornamentation and reproduction (Fitzpatrick, Berglund & Rosenqvist 1995). Females that invest in ornamentation may be less able to invest in reproduction than females of equivalent quality that invest only in offspring (Fitzpatrick *et al.* 1995; Berglund, Rosenqvist & Bernet 1997). Thus, the reduction in the investment in reproduction associated with an enhanced expression of female traits can lead males to avoid heavily ornamented females (Chenoweth, Doughty & Kokko 2006). Females are expected to invest in ornamentation when the fitness benefits of the ornaments are higher than the direct investment into the offspring (LeBas, Hockham & Ritchie 2003). From an evolutionary perspective, male mate choice is not the only force that can explain the presence of these female flashy traits, but they can also arise due to a shared genetic architecture between males and females (Lande 1980) or due to Social Selection (Tobias, Montgomerie & Lyon 2012; see section: "*social competition*").

Age

Many animals have their life divided in three major stages: growth, when they depend on parental care of some sort; immaturity, when they become independent from their parents; and adulthood. During these stages, signalling expression changes due to the different selection pressures at each stage, caused by variation in the trade-offs associated with signalling. During growth, the major role of signalling is involved in the communication between parents and offspring (Searcy & Nowicki 2005), particularly with begging calls of the offspring. Begging can be described as the behaviour of food solicitation from the offspring to the parents, and involves different behavioural and signalling traits that allow parents to evaluate the nutritional requirements of the chicks (Welty 1975; Redondo & Castro 2010). Immature signalling can signal subordination to adults in order to avoid agonistic encounters, like for example when juvenile birds do not exhibit adult plumages (delayed plumage maturation; Lyon & Montgomerie 1986). In addition, they can also work as signals of status, modulating the access to limited resources within immature individuals, in general, and siblings, in particular (Jones 1990; Vergara & Fargallo 2008). Finally, during adulthood the two-main contexts where signalling works are mate choice and resource competition, when individuals use signals to evaluate the quality of the potential mates (Andersson 1994) and the competitive abilities of the opponents (Rohwer 1975), respectively. As adulthood can last for several years, the optimal allocation of resources to ornamentation changes as individuals age (Stearns 1992). Life-history theory allows us to understand how these changes are modulated by Natural Selection. The central point of life-history theory is that the amount of resources available to an individual is limited and thus individuals have to choose whether to devote them to the different life-history traits, mainly reproductive- or survival-related, that will enhance their fitness (Stearns 1992). Therefore, it is straightforward to think that the resources that an individual has can come into conflict between devoting them to afford the cost of ornamentation as suggested above, or to ensure the success on different life-history traits. The general age-dependent pattern includes an increase in signalling during early life,

followed by a peak in mid-life and late-life deterioration (senescence). However, senescence can be difficult to find in the wild due to the weakening of Natural Selection in late-life (Nussey *et al.* 2013). Most studies exploring the effects of senescence in reproduction or ornamentation have usually focused on long-lived species like the Asian elephant (*Elephas maximus*; Robinson, Mar & Lummaa 2012a) or the wandering albatross (*Diomeda exulans*; Froy *et al.* 2013), however, finding evidences of those effects on short-lived individuals is not an easy task (Turbill & Ruf 2010).

From an evolutionary perspective, there are three different theories for the evolution of senescence (Monaghan *et al.* 2008). In 1977 Thomas Kirkwood, based on some ideas previously presented by August Weissmann (1891), proposed the disposable soma theory (Kirkpatrick & Ryan 1991). This theory is centred in the trade-off between devoting limited resources to self-maintaining and other activities, like reproduction. When individuals devote resources to other activities rather than to self-maintaining, they suffer from somatic damage that is accumulated as they age, causing a reduction in performance in late-life (Westendorp & Kirkwood 1998; Sgrò & Partridge 1999). The second idea states that senescence is caused by a decline in the strength of selection at late-life, that will allow deleterious mutations that act at late-life to accumulate (mutation accumulation theory; Medawar 1952). Finally, in 1957 George C. Williams proposed the agonistic pleiotropy theory, defending the existence of genes with positive effects in early-life that are deleterious at late-life. The presence of these genes will be favoured by selection due to their contribution to the survival and reproduction of young individuals in spite of the noxious effects in old individuals (Williams 1957).

Different studies have reported the age-dependence of different traits in wild populations (Reimers, Holmengen & Mysterud 1999; Reid *et al.* 2003; Carranza *et al.* 2004; Jones *et al.* 2008), being life-history traits those that have received more attention (Jones *et al.* 2008). In general, the current knowledge about the age-dependence of signalling traits is scarce, although current evidence shows that the expression of many traits changes with age in multiple species (Torres & Velando 2003; 2007; Galván & Møller 2009; Forstmeier *et al.* 2006). However, many of these studies do not follow a within-individual approach and thus their results may be confounded, as demographic heterogeneity can hide the individual-level trajectories of signal expression (van de Pol & Verhulst 2006; Vaupel & Yashin 1985; van de Pol & Wrigth 2009). The number of studies tackling the age-dependence of signalling traits following a within-individual perspective are scarce, perhaps due to the difficulties related to obtaining the longitudinal datasets required by this approach (Nussey *et al.* 2008). The patterns shown by those studies evidence within-individual increases in trait expression with age, in both males and females of different species (Candolin 2000; Miller & Brooks 2005; Evans, Gustafsson & Sheldon 2011; Evans & Sheldon 2013; Potti, Canal & Serrano 2013). These results rather neglect the effects of senescence in the expression of coloured secondary sexual traits, and can be better framed under a life-history context where individuals increase their investment in reproduction as their residual reproductive value diminishes (Kokko 1997). Alternatively, other studies have been able to find senescent patterns in the expression of sexual traits

like the size of the comb in black grouse (*Tetrao tetrix;* Kervinen *et al.* 2015) or the length of the tail feathers in barn swallows (Balbontín *et al.* 2011; reviewed in Lemaître & Gaillard 2017). These divergences in the ageing patterns of secondary sexual traits evidence the need of more studies to unravel the factors modulating the age-dependence of ornamental traits. Of special interest are the potential effects of environmental conditions on the age-dependence of ornamental signalling and their reproductive consequences (Lemaître & Gaillard 2017). Different studies have shown that environmental heterogeneity can modulate different aspects of ornament expression, like the allocation priorities of the individuals (Martinez-Padilla *et al.* 2010; Vergara & Martínez-Padilla 2012; Martínez-Padilla *et al.* 2014). However, the environmental effects on the age-dependence of ornamental traits remain unexplored (Lemaître & Gaillard 2017), despite being a cornerstone to fully understand the patterns of signal expression throughout the life of individuals.

(b) Environmental constrains

In spite that the role of the above-mentioned individual intrinsic factors is striking, the influence of other factors associated with environmental variation cannot be ruled out. Most animals inhabit heterogeneous environments that fluctuate in time and space and may modulate signal expression. One example that perfectly illustrates this situation are colouration patterns in the European map butterfly (*Araschnia levana*; Gilbert 2005). This species shows high variability in the expression of the colouration in the wings. The variation is so extreme that it led Linnaeus to originally classify them in different species. Nowadays, however, it is known that environmental heterogeneity is behind such variation, specifically the variations in temperatures and photoperiods experienced by butterflies during early pupation (Gilbert 2005). Fluctuations in the environmental conditions can, not just modulate the expression of animal colouration and their condition-dependence (Vergara *et al.* 2012a), but also determine other important factors like breeding densities or reproductive success (Fargallo *et al.* 2009; Korpimäki & Norrdahl 1991). Therefore, studying the effects of environmental heterogeneity is crucial to fully understand the function of signalling traits and the patterns behind their expression.

Environmental conditions

As explained above (in Section 2), one of the fundamental ideas behind the evolution and maintenance of effective communication systems is that the reliability of the signals is obtained by the cost of their production or maintenance (Zahavi 1975). For instance, the expression of certain traits can be constrained because they are produced with elements that are limited in nature and have other non-signalling functions, like for example carotenoid-based traits (Pérez-Rodríguez 2009). When it comes to carotenoid signalling, individuals have to decide whether to invest the carotenoids in the signalling traits or devote them to other physiological functions (Schantz *et al.* 1999). Alternatively, the cost

can be caused by other factors not associated with the production of the trait. For instance, the honesty of some melanin-based traits is associated with maintenance costs as the individuals bearing enhanced versions of the trait face increased aggression levels by conspecifics (Rohwer 1977; Rohwer & Rohwer 1978). However, these costs are not fixed and can be modulated by environmental heterogeneity. Several studies have shown that environmental conditions can determine the expression of different ornamental traits in both males and females (Griffith, Owens & Burke 1999; Chaine & Lyon 2008; Martinez-Padilla *et al.* 2010; Robinson *et al.* 2012b; Vergara & Martínez-Padilla 2012; Vergara *et al.* 2012b; c; Martínez-Padilla *et al.* 2014). For example, in male red grouse (*Lagopus lagopus scoticus*), climatic conditions and parasite load are negatively related to the expression of a secondary sexual trait, the size of the supraorbital comb (Vergara *et al.* 2012c), or the costs associated with its expression (Martinez-Padilla *et al.* 2010). Secondary sexual traits are condition-dependent when the expression of the signal reliably shows the individual quality of the bearer (Morehouse 2014). Further, environmental conditions can also modulate the condition-dependence of this same secondary sexual trait (Vergara *et al.* 2012a; Martínez-Padilla *et al.* 2014). In general, harsh environmental conditions are expected to be associated with more tight condition dependence of the trait. In other words, under tough conditions, only individuals in prime quality will be able to express high-quality signals, because only high-quality individuals can afford the costs of expressing more elaborate traits. Otherwise, under benign environments the condition dependence is weakened, and both high- and low- individual quality will be able to express high-quality signals. This emphasizes the mediating effect of environmental conditions, not only as modulators of signal expression, but also determining the reliability of signalling traits (Higginson & Reader 2009; Vergara *et al.* 2012a). From a life-history perspective, is interesting to point out that environmental conditions can also modulate the allocation priorities in different trade-offs where signalling is involved (Badyaev & Duckworth 2003; Gustafsson, Qvarnström & Sheldon 1995). Factors like social environment can modulate the allocation priorities of the individuals (Martinez-Padilla *et al.* 2010; Vergara & Martínez-Padilla 2012; Vergara *et al.* 2012c; Tarjuelo, Vergara & Martínez-Padilla 2016). For example, in areas where intra-sexual competition was experimentally increased, male red grouse calling behaviour was shorter than calls made by males living in areas with un-manipulated levels of intra-sexual competition (Tarjuelo *et al.* 2016).

These studies stress the relevance of the environmental conditions on the expression of animal signals, either social or sexual. However, our knowledge of how environmental heterogeneity influences the expression of certain traits is scarce. In addition, there is also a lack of information on how environmental conditions modulate the associations of signalling traits and individual fitness. Finally, it is also interesting to point out that environmental factors can interact between them. For example, it has been shown that breeding density can be positively associated to food availability (Fargallo *et al.* 2009), thus, under more benign environmental conditions we can also expect increased competition levels. In addition, these interactive effects can also occur between environmental and non-environmental factors. For instance, it has been shown that

environmental conditions can modulate senescence rates for survival probability (Holand *et al.* 2016). However, there is a lack of studies exploring the interactive influence of environmental conditions and age-dependence on the expression of signalling traits.

Social competition

Social competition is a major selective force in natural populations, and mediates the expression of different signalling traits (Tobias *et al.* 2012). It was in the second half of the 20th century when the role of competition for non-reproductive resources was considered as a selection pressure similar to Sexual Selection (Wynne-Edwards 1962; Crook 1972; Ghiselin 1974). Based on these studies, in 1983 Mary Jane West-Eberhard proposed the theory of Social Selection (West-Eberhard 1983), suggesting that individuals have differential reproductive success due to a between-individual variation in social competition for any kind of resource (West-Eberhard 1983). The original vision of West-Eberhard included competition for reproductive resources (mating) and according to this, Sexual Selection is a type of Social Selection where individuals compete for access to mates. Nowadays, however, the term "Social Selection" is mainly used in competition contexts for non-reproductive resources (Tobias *et al.* 2012).

Within a social competition context, animals compete for limited resources like food or breeding sites (Huntingford & Turner 1987). The output of among-individual interactions is usually biased to a small number of individuals that have preferential access to food or any other resource. In 1922, Thorleif Schejelderup-Ebbe described this asymmetry introducing the concepts of social hierarchies and dominance relationships (Schejelderup-Ebbe 1922). These hierarchies determine the access to limited resources avoiding the agonistic encounters. However, how does an individual know the status of a potential rival? In 1975, Sievert Rohwer proposed that the winter plumage of different avian species signalled the individual status within a social hierarchy (Rohwer 1975). The presence of these signals, known as badges of status, allows individuals to recognise the competitive abilities and therefore, the status of potential contestants, avoiding agonistic encounters (Dawkins & Krebs 1978). Thus, badges of status benefit both dominant and subordinate individuals as they reduce the number of aggressive encounters and their associated costs (Rosvall 2011). Despite that the system of status signalling has been shown in different species, one aspect that remains less clear is the honesty of these signals of social status. In other words, do these signals reliably transmit a message of individual quality?

The stability of this signalling system has been explained by different hypotheses (Senar 2003). First, the "*sceptical receptor hypothesis*" states that status signals are expected to be reliable when they have an associated consistent behaviour. This will allow receptors to focus not only on the signal but also on the way individuals behave, ensuring the reliability of the received signal (Rohwer 1977; Järvi, Walsø & Bakken 1987; Rohwer & Rohwer 1978). Second, the reliability of the badges of status can be achieved by the costs associated with maintaining the trait, rather than producing it. These costs will arise from

increased intra-sexual aggressiveness (social control hypothesis; Rohwer 1977; Rohwer & Rohwer 1978), predation (differential predation hypothesis; Fugle *et al.* 1984; Fugle & Rothstein 1987) or immunosuppression, since only individuals in prime quality will be able to stand the immunosuppressive effects associated with the hormones that regulate the expression of those signals (immunocompetence handicap hypothesis; Folstad & Karter 1992). Third, in 1982 John Maynard-Smith proposed "*the mixed stable evolutionary strategy hypothesis"*, stating that both dominant and subordinate individuals may follow alternative but evolutionary stable strategies (Maynard Smith 1982). These divergent strategies are evolutionary stable because dominant and subordinate individuals maximize their fitness by behaving as dominant and subordinate. Within this context, subordinate individuals are not expected to use false signals to increase their position in the dominance hierarchy, because they are already maximizing their fitness being subordinate, and if they did cheating will be penalized. This may be achieved, for example, when dominant and subordinate individuals allocate their resources differently, trading-off resources to obtain high-quality territories and parental care (Studd & Robertson 1985, but see Yezerinac & Weatherhead 1997). However, the vast proportion of the studies exploring the role of the badges of status have been focused on males, leaving the expression of these signals in females underexplored (Tobias *et al.* 2012). Recent studies have evidenced that females, like males, exhibit traits that are used as badges of status in intra-sexual competition contexts (Murphy *et al.* 2009; Midamegbe *et al.* 2011; Morales *et al.* 2014; Crowhurst *et al.* 2012; Tobias *et al.* 2012). However, in spite of these studies showing the role of signals of status in females, there is a lack of information about the factors that modulate their expression and on which contexts these traits are used.

Social competition is not exclusive of the adult age-class, and individuals during the first stages of life also compete between them. It is well known that, in many species, offspring develop different signals conveying information about their levels of need (Kilner 1997) or their quality (Thorogood *et al.* 2008), that are used by parents to adjust the levels of parental care (Lyon, Eadie & Hamilton 1994; Kilner 1997; Jourdie *et al.* 2004). However, the information available about the signals used just after the nestling stage, during the post-fledgling dependence period (PFDP), is less abundant. The PFDP is defined as the period between fledglings first flight and the time when they become totally independent from their parents (Mock & Parker 1997). From a general perspective, signalling during the PFDP can work in two different contexts. On the one hand, offspring signals can work in a parent-offspring context, signalling nestling demand and/or nestling quality (Penteriani *et al.* 2007; Siefferman *et al.* 2008; Moreno & Soler 2011). For example, in eagle owls (*Bubo bubo*) the brightness of the feathers surrounding the mouth modulates the amount of food received by the fledglings during the PFDP (Penteriani *et al.* 2007). On the other hand, offspring signalling traits can work within the fledgling age class conveying information about the competitive abilities of the bearers (Jones 1990; Vergara & Fargallo 2008; Moreno & Soler 2011; Fargallo *et al.* 2014). For instance, fledglings of male common kestrels (*Falco tinnunculus*) bearing greyer rumps are more successful than those with browner rumps, when competing for preys provided by the parents (Vergara

& Fargallo 2008). In this context, these signals could work as badges of status modulating the access to the parental feedings without any agonistic encounter. From a mechanistic perspective, two different hypotheses have been proposed to explain the length of the PFDP. The "*resource competition hypothesis*" states that fledglings will try to remain dependent as much as they can to increase the amount of received parental care (Vergara, Fargallo & Martínez-Padilla 2010). Alternatively, the duration of the PFDP has been explained by the "*ontogenetic hypothesis*", according to which fledgling independence occurs as soon as they reach an optimum body condition (Ferrer 1993; Muriel *et al.* 2015). Regardless of the mechanism behind the duration of the PFDP, this period is of crucial importance for fledglings. During the PFDP, fledglings benefit from the care provided by the parents to improve their foraging and flying skills (Davies 1976; Marchetti & Price 1989; Wheelwright & Templeton 2003; Wheelwright, Tice & Freeman-Gallant 2003; Yoda, Kohno & Naito 2004). During the PFDP, offspring improve their nutritional status, which is a determinant factor modulating offspring survival once they reach full independence (Bouwhuis *et al.* 2015; Perrig *et al.* 2017). These studies highlight the role of the length of the PFDP for the interests of the offspring, that will benefit from effectively communicating with both their parents and siblings. Still, the information about the fitness consequences of the expression of these signals during the PFDP is scarce, perhaps due to the difficulties of monitoring young birds once they fledge (Langen 2000).

The studies shown above evidence that the use of morphological traits, like badges of status, as signals expressed to assess potential rivals are abundant. Several studies have explored the association of individual personality and social dominance, finding a general trend showing that proactive individuals become dominant more often. Animal personality is defined as the inter-individual differences in behaviour consistent across time and context (Sih, Bell & Johnson 2004; Réale *et al.* 2007; Stamps & Groothuis 2010; Griffin, Guillette & Healy 2015). Personality is defined using 5 different axes, including: activity, shyness-boldness, exploration-avoidance, aggressiveness and sociality (Réale *et al.* 2007) that can be correlated between them (Huntingford 1976; Coleman & Sloan Wilson 1998; Koolhaas *et al.* 1999; Biro & Stamps 2008). Personality is an important trait for individuals, and different studies have shown that its variation is positively associated with fitness (Cote & Clobert 2007; Boon, Réale & Boutin 2007; van Oers *et al.* 2008; Réale *et al.* 2009). For example, a study using North American red squirrels (*Tamariscus hudsonicus*) showed that those offspring with more active and aggressive mothers had higher growth and winter survival rates, respectively (Boon *et al.* 2007). This association between fitness and personality suggests that this trait can be under selection. Animal populations are composed by individuals with different personalities. From an evolutionary perspective, the maintenance of this behavioural diversity can be explained by the trade-offs faced during life (Sih *et al.* 2004; Wolf *et al.* 2007; Stamps 2007). For instance, bold individuals usually have increased reproductive success but they also incur in higher mortality rates when compared to shy individuals (Smith & Blumstein 2007).

c) Interaction between individual characteristics and environmental factors

As briefly explained above, the factors modulating signal expression do not act independently and can interact among them. Environmental effects on the expression of social or sexual signals can modulate, for example, the allocation priorities to signalling of individuals at different ages. Since the energy available to a certain individual is limited, it faces an important decision in each breeding season, whether to invest in survival and somatic maintenance or to invest in reproduction (Stearns 1992), being this dichotomy one of the most classical trade-offs described in nature. Within this context, life-history theory predicts that the investment in reproduction should be negatively correlated to the individuals' residual reproductive value (Kokko 1997). Thus, according to the theory, it can be expected an increase in ornamentation as individuals age (Kokko 1997). However, the resolution of this trade-off can differ depending on the contexts when the signal is displayed (Badyaev & Duckworth 2003; Martinez-Padilla *et al.* 2010). For example, the association between age and ornamentation in house finches (*Carpodarcus mexicanus*) is modulated by pairing status the previous reproductive season, being those finches that were single the ones that invest more in ornamentation the following reproductive season (Badyaev & Duckworth 2003). The interactive effects of the factors modulating signal expression have been reported for the action of parasites on the association between ornamentation and testosterone (Martinez-Padilla *et al.* 2010). Still, there are many questions about the potential interaction between the factors that modulate signal expression that remain unravelled.

In this section, we have shown that signalling traits used in different communication contexts can be modulated by different factors, both intrinsic and extrinsic to the individuals. In addition, we have also shown that these factors do not act alone and that can interact between them. However, not all signals used in animal communication are equally sensitive to the fluctuations of the same factors. Some traits are under a higher genetic control compared to others that are modulated by environmental forces, influencing the sensitivity of the signal to the action of intrinsic or extrinsic factors. In this thesis, I have studied the expression of melanin-based traits. Originally, it was thought that these traits were genetically determined and that the role of the environment on their expression was rather subtle (Roulin & Dijkstra 2003; Niecke, Rothlaender & Roulin 2003). Nowadays, the genetic influence on the expression of melanin-based traits has been reinforced, but it is clear, at the same time, that environmental conditions do mediate the expression of these signals (Fargallo *et al.* 2007; Potti & Canal 2011; Kim *et al.* 2013). This duality makes melanin-based traits especially suitable for the study of how individual characteristics, environmental variability, and the interaction between them modulate their expression.

4. MELANIN-BASED COLOURATION

One of the most widespread forms of signalling in the animal kingdom is the display of colourful patches or structures. Among terrestrial vertebrates, birds are the most coloured animals, with plumages representing almost all imaginable colours (Stoddard & Prum 2011). This diverse communication system has evolved in response to the avian visual system (Hart & Hunt 2007). Most birds have a tetrachromatic vision, with 4 cone types, that allows them to perceive a wide range of colours, from the red to the ultraviolet wavelengths (Cuthill *et al.* 1999; Hart 2001; Jones & Osorio 2004). Bird colouration is formed by two different mechanisms that can work together. Firstly, by the interaction between light and the nanostructure of the feather (structural colouration), like for example in the iridescent gorget present in many hummingbirds (Family: *Trochilidae*; Stoddard & Prum 2011). Alternatively, bird colouration can be produced by the deposition of pigments on the feathers that absorb certain wavelengths (pigmentary colouration), and reflect particular colours. The main used pigments are melanins and carotenoids (Hill & McGraw 2006) that can produce different kinds of colourations, like the black bib of house sparrows (*Passer domesticus*; McGraw, Dale & Mackillop 2003) or the red colouration in the house finch (Hill 1992), respectively.

Melanin is the most common pigment in animal integuments, being usually the pigment responsible for the base colouration in many animals (McGraw 2006). Melanin-based colourations are the product of two different molecules, eumelanin and pheomelanin, that are synthetized in a process known as melanogenesis (Ito & Wakamatsu 2008; Fig.1.2). It has been proposed that melanogenesis is controlled, at least in part, by the melanocortin system that also includes a plethora of other physiological pathways involved in different activities unrelated to melanin synthesis (Cone 2006). For instance, the melanocortin receptor 5 (MC5R) is a component of the melanocortin system that regulates different physiological functions associated with aggressiveness (Morgan & Cone 2006; Cone 2006).

Melanin-based traits have a prominent role in the animal communication within a social context (Jawor & Breitwisch 2003; Senar 2006). Different studies have evidenced the role of melanin-based traits as badges of status (Rohwer 1975; Nakagawa *et al.* 2007; Senar *et al.* 1993; Tibbetts 2006). For example, in 1975 Sievert Rohwer showed that the black plumage of the Harris sparrows (*Zonotrichia querula*) works as a dominance signal during winter, with dominant individuals exhibiting darker plumages (Rohwer 1975). However, melanin-based traits not only work within a social context but also as indexes of quality within a Sexual Selection context (Roulin *et al.* 2000; Parker *et al.* 2003). Many studies have shown that the expression of melanin-based traits differs between females and males and, in some cases, an enhanced expression of melanin-based traits can be positively selected by females, like for example, in common kestrels (Palokangas *et al.* 1994) or Kentucky warblers (*Oporornis formosus*; Parker *et al.* 2003). Melanin is the main responsible for most of the coloured patterns found in animals (Pérez-Rodríguez, Jovani & Stevens 2017). These patterns can be formed by spots, whose size and number

can be under different selection pressures, transmitting different information to receivers (Roulin 2016). It is also interesting to point out that recent research evidences that the patterns formed by these melanin-based traits may act as indexes of individual quality (Pérez-Rodríguez *et al.* 2017), suggesting that the role of melanin-based traits as proxies of individual quality is more extended than previously thought. This has been reaffirmed by a recent review compiling the different costs associated with melanin-based signalling, stressing that the honesty of expressing melanin-based traits can be ensured by different physiological and behavioural processes (Roulin 2015).

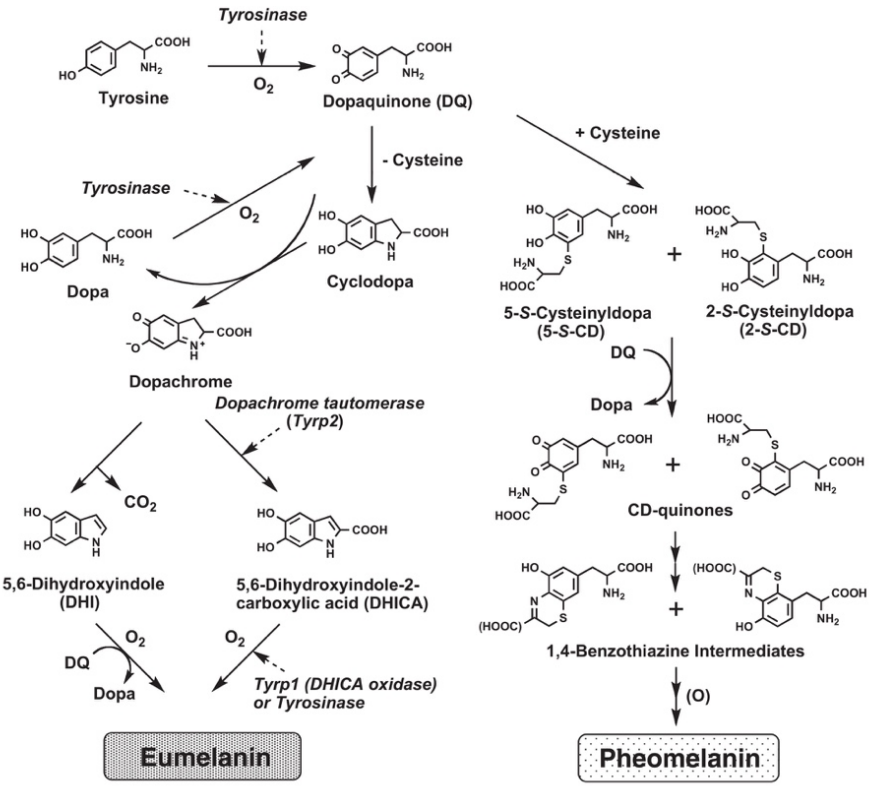

Figure 1.2: Schematic representation of the melanogenesis pathway for eumelanin and pheomelanin. Adapted from Ito *et al.* (2008).

One of the most important controversies that have surrounded melanin-based colouration is whether its expression is sensible to the environmental variation or not. This debate probably roots on the fact that, unlike other pigments like carotenoids that have to be acquired through the diet (Olson & Owens 1998; Pérez-Rodríguez 2009), melanin can be synthetized *de novo* by the individuals. Nowadays, it is known that in spite of the strong genetic basis of the melanin-based colourations (Roulin & Dijkstra 2003; Hubbard *et al.* 2010; Kim *et al.* 2013), the role of environmental conditions modulating their expression is inarguable (Veiga & Puerta 1996; Griffith *et al.* 1999; Fitze & Richner 2002; Fargallo *et al.* 2007; Vergara *et al.* 2009). Three of the main environmental effects that have been reported to modulate the expression of melanin-based traits are, rearing conditions (Griffith *et al.* 1999), diet (Veiga & Puerta 1996; Fargallo *et al.* 2007) and parasites (Fitze & Richner 2002). The effects of rearing conditions showed that the size of the badge of sons was correlated to the size of the badge of foster parents rather than to the one of the

genetic father (Griffith *et al.* 1999), evidencing that the expression of this melanin-based trait is modulated by the environment where chicks grow. When it comes to parasites and diet, different studies have shown that under high food and low parasite scenarios individuals exhibit enhanced melanin-based traits (Veiga & Puerta 1996; Fitze & Richner 2002; Fargallo *et al.* 2007). However, much research is needed to shed light on different aspects of melanin-based colouration that remain unexplored. There is lack of information about the role of this kind of colouration on other contexts, like within female intra-sexual competition or in the signalling during the first stages of life.

5. STUDY SPECIES – THE COMMON KESTREL

My study species is the common kestrel (hereafter kestrel). Kestrels have a Palearctic, Afrotropical, and Indomalayan distribution. In Spain, they are present along all the peninsula and in the Canary and Balearic Islands (Village 1990; Martínez-Padilla 2004). Unlike northern populations that migrate south during winter, Spanish populations perform partial migration movements after breeding. The breeding cycle of kestrels starts between March and April when they arrive to the breeding grounds and start pairing up. Similarly to other raptors, male kestrels perform courtship feedings during the pairing period that continues during nearly the whole breeding season (Village 1990; Mougeot, Thibault & Bretagnolle 2002). As courtship advances, males feed his mate more frequently and females spend less time hunting for herself, improving female body condition (Village 1990). Egg laying usually starts in mid-April, but it variates depending on the individuals and on the environmental conditions (Aparicio 1998). In our population, female kestrels lay a mean of 5.00±0.03 eggs (range: 2-7; n=613), in a two-day interval (Martínez-Padilla & Viñuela 2011).

Incubation takes approximately 28 days and is performed mainly by females. During this stage, the role of males consists in feeding the female and sporadically incubating the eggs (Village 1990). Nestling stage has a mean duration of 31 days (Vergara & Fargallo 2008). During the nestling period, there is a divergence in the role of both parents. During the first two weeks, males are the main responsible of providing food and females deliver the preys obtained by the male to the nestlings and split them among the chicks. After the half of the nestling period, females start helping males with food provisioning and instead of splitting the preys among their nestlings, she just leaves the prey intact inside the nest and nestlings eat it by themselves (Village 1990). During this stage, due to the size dimorphism, females are 4% bigger than males, which is appreciable since chicks are 11 days old, thus females have a competitive advantage on their male siblings (Fargallo *et al.* 2002; 2003 but see Martínez-Padilla & Viñuela 2011), resulting in better body condition (Dijkstra *et al.* 1990; Wiehn & Korpimäki 1997; Fargallo *et al.* 2002).

Once nestlings leave the nest, the post-fledgling dependence period (PFDP) starts. In one of the study sites (CA; see below), the mean number of fledglings produced by a pair is 3.66±0.07 (range:0-7; n=613). During this stage, fledglings remain in the surrounding of the nest and still depend on their parents for food and protection. In CA (see below), the mean duration of the PDFP is 15.25±0.40 days (n=315). However, all siblings do not become independent at the same time, showing a within-brood variation of 6.63±0.60 days (n=86).

Kestrels show a reversed sexual dimorphism in size, where females are approximately 20% larger than males (Village 1990). This species is also dimorphic in their plumage and males exhibit a more colourful and conspicuous plumage than females (Village 1990). Females are brown in the head, body, and wings with black bars regularly distributed in these areas. Rump colouration can change within and between individuals and it ranges from being completely brown to completely grey, with a variable number of black bars on it (Fig.1.3). Female tails are usually brown with black bars, but they can show variable amounts of grey colouration (Fig.1.3). Males exhibit a red-brownish plumage with black spots. Males head, rump and tail display a grey unbarred colouration. Fledgling display colourations similar to those exhibited by females, and they also display variable amounts of grey in the rump, being the expression of this trait more common in males than in females (mean ± se percentage of grey in the rump: males 23.60±0.72 n=778; females 2.28±0.71 n=786).

Kestrels replace their feathers every year, where adult moult is timed partly to calendar and partly to the start of breeding (Village 1990), taking place between the months of May and October. Breeding females usually begin to moult during incubation when they have low energy expenditure, although moulting can stop when they start helping males to acquire food to feed the offspring. Male moult takes place later in the breeding cycle when their energy demands decrease (Village 1990).

Kestrels are generalist predators that feed in a wide diversity of species (Navarro-López, Vergara & Fargallo 2014; Village 1990). Small mammals are the main food item in the kestrel diet, representing the 49.7% of the biomass in their diet (Navarro-López *et al.* 2014). Within mammals, kestrels feed particularly on common voles (*Microtus arvalis*; 48.5% of the biomass of their diet, Navarro-López *et al.* 2014) whose densities are associated with kestrel population size and climatic variation (Fargallo *et al.* 2009). In addition, kestrels also feed on reptiles, arthropods and other species of birds or amphibians (Fig. 1.4; Navarro-López *et al.* 2014).

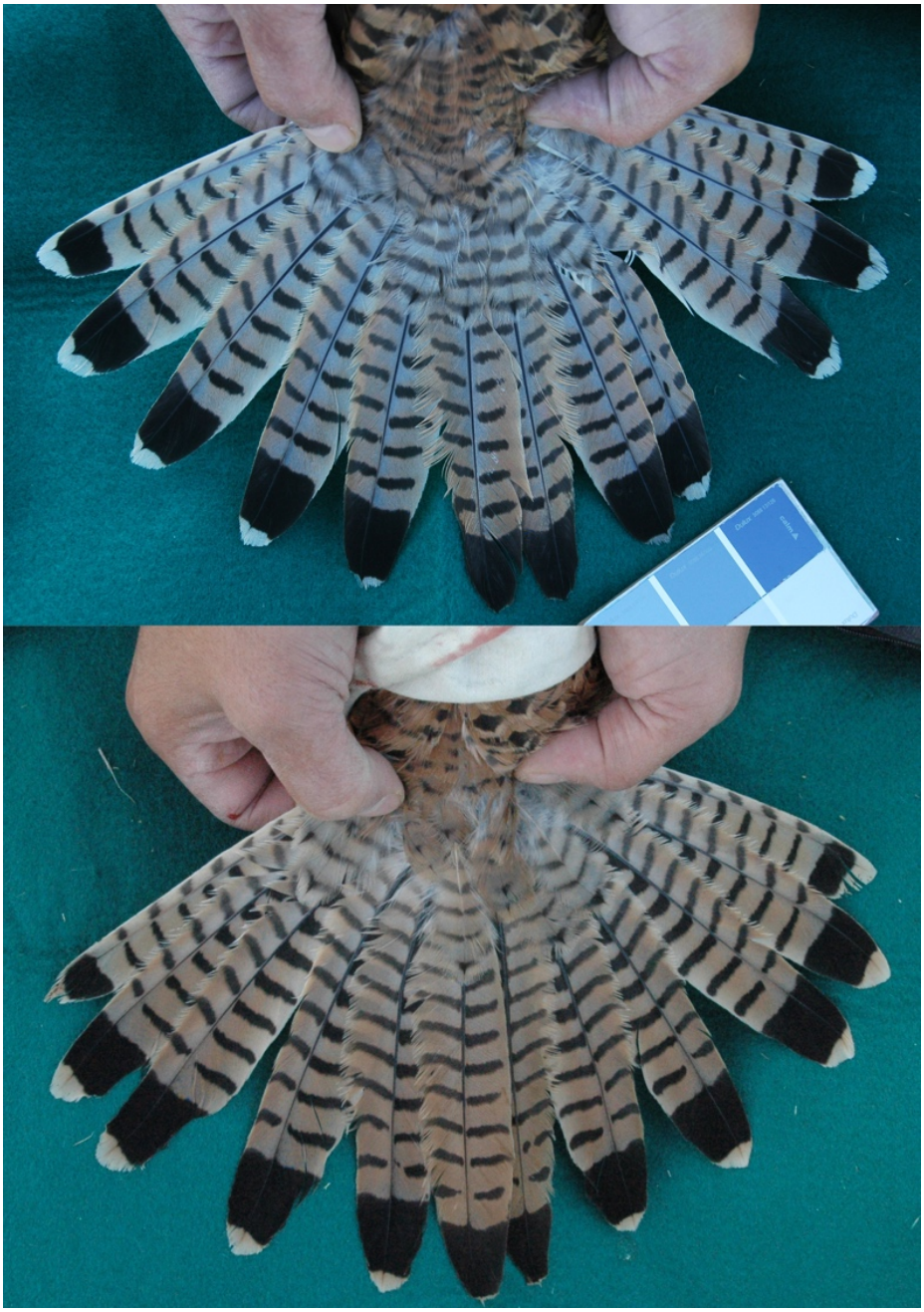

Figure 1.3: Variability in the grey colouration present in the rump and the tail of two adult female common kestrels.

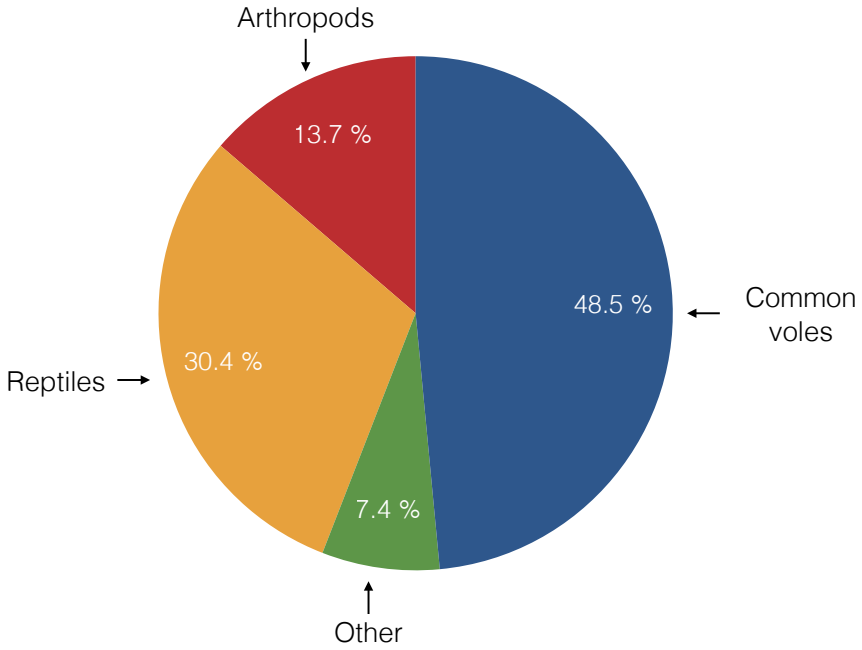

Figure 1.4: Percentage of biomass of the prey items delivered by parents to offspring between 2006-2009. Data taken from Navarro-López *et al.* (2014).

6. STUDY AREAS

The field work of this dissertation was carried out in two different areas of the region of Castilla y León: Campo Azálvaro (CA) and Villalar de lo Comuneros (VC). All studies performed in the thesis include data of CA and, in addition to the data taken by myself from 2013 to 2016, in Chapters I and II, I also include data taken by Juan Antonio Fargallo, Jesús Martínez-Padilla, Pablo Vergara, Isabel López-Rull and Juan Navarro-López from 2002 to 2013. VC was included as an extra population during the year 2014 in order to increase the number of observations and the environmental variability during the experiment performed in Chapter III.

(a) Campo Azálvaro

Campo Azálvaro region is located in the Central System in the provinces of Segovia and Ávila (40º40´N; 4º20´W) at 1300 meters a.s.l. This area is a mountainous grassland, mainly devoted to cattle-rising crossed by the river Voltoya (Fig. 1.5). In CA, there are 62 nest boxes that have been installed progressively from 1994 to 2005 (Fargallo *et al.* 2001). The population has been under a long-term and individually-based monitoring since then, where all breeding individuals and their offspring are captured and individually marked each year. The number of breeding pairs in the area from 2005 to 2016 varies between 22 to 44 pairs, with an average of 31 pairs.

Figure 1.5: Campo Azálvaro (Segovia)

(b) Villalar de los Comuneros

Villalar de los Comuneros is located in the region of Valladolid (41º32´N; 5º08´W) at 700 meters a.s.l. and at about 115 km away from CA (Fig. 1.6). VC is a cropland area with a mixture of both irrigated and non-irrigated crops. In the area, there are 100 nest-boxes installed in 2009 as an exploratory method to control common vole populations after a major outbreak in 2007 (Paz *et al.* 2013). Population size between 2012 and 2014 ranged from 26 to 64 with a mean number of 39 breeding pairs.

Figure 1.6: Villalar de los Comuneros (Valladolid).

7. POPULATION MONITORING

In both populations, the approaches to monitor the breeding season were equivalent. Nest monitoring began in mid-April, when all nests were visited every two days in order to detect laying date (day the first egg is laid) and clutch size. Females were captured twice. First, during egg incubation using a net and a second time, when we captured the males, using a trap when the nestlings were 10-13 days old. All breeding adults were ringed using metal rings and since 2013 also with a PVC ring bearing a unique alphabetic code. During the captures, adult individuals were identified as yearlings or adults according to the ring code if they were recaptured, or by plumage features when they were not ringed (Vergara & Fargallo 2007). With an age of 25 days, nestlings were blood sampled for molecular sexing, and ringed using metal rings. In addition, during the year 2012 nestlings were marked with a unique combination of colour rings, after that and since 2013 onwards, nestlings were marked with the same PVC rings than adults. During the captures, we measured wing, tarsus size (to the nearest mm) and weight (to the nearest g) of both adult and offspring. We also estimated the percentage of grey colouration by sight (0 corresponded to completely brown rumps and 100% to completely grey rumps) in the rump or both adult females and offspring, a measurement that has proven to be high repeatable (for further information see Vergara *et al.* 2009).

Associated to the long-term monitoring of the kestrel population, we also assessed the abundance of common voles by two trapping sessions per year, in autumn and spring in CA. Abundance of voles have been carried out every autumn and spring since 1997 (Fargallo *et al*. 2009). The trappings were carried out by setting one-hundred Sherman traps in four plots (25 each) that were monitored six times for three days, half of them at down and the other at dusk (Fargallo *et al.* 2009).

Common kestrels breeding in our study sites represent a perfect system to tackle the objectives considered in this thesis for several reasons. Firstly, in both study areas the great majority of the kestrels breed in nest-boxes, allowing us to precisely determine both the number of breeding pairs and their reproductive output. Second, the landscape of both study sites constitutes a perfect place to assess behavioural traits from long distances, as both CA and VC are mainly plain and treeless. Third, kestrels display different melanin-based trait that present within- and between-individual variations. Finally, the opportunity to monitor the abundance of voles, an important component of kestrel diet, allows us to study the mediating role of this environmental factor in the expression of melanin-pigmented traits.

8. OBJECTIVES

The pivotal point and general aim of this thesis is the study of the environment-mediated influence on the function and fitness benefits associated with the expression of melanin-based colouration in both young and adult kestrels. This PhD thesis is organized in 5 chapters with their own particular objectives:

(i) In the first chapter, I aim describing the within-individual age dependence of kestrel spottiness, a trait that has been described to work in a Sexual Selection context (Palokangas *et al.* 1994). I used two different characteristics of black spots, their number and size, and how they changed during the life of males and females separately. In addition, I aim exploring the interactive effect of age and vole abundance on the expression of the number and size of the spots.

(ii) In the second chapter, I aim exploring the within-individual variation of female rump colouration. Further, I studied the effect of environmental conditions on the expression of this trait and its association with female fitness.

(iii) In the third chapter, I aim studying the role of female rump colouration within an intra-sexual competition context. I did so by means of natural decoys of kestrels exhibiting totally grey or totally brown rumps. I used those "phenotypes" because a previous study suggests that the proportion of grey colouration in the rump is an index of quality in female kestrels (Vergara *et al.* 2009).

(iv) In the fourth chapter, my objective was to study how the duration of the post-fledgling dependence period modulates fledgling recruitment probability. In addition, as previous studies have shown that offspring rump colouration is an index of their competitive abilities, I also explored how the expression of this trait mediated the association between the length of the PFDP and recruitment. Finally, as parental care is expected to be costly for the parents, I studied the association between duration of the PFDP, and parental reproduction and survival in the following breeding season.

(v) In the fifth chapter, I aim exploring whether individual signalling can be a reliable proxy of individual behaviour in nestlings. Specifically, I studied the association between melanin-based colouration of nestlings and their personality. I did so by performing different behavioural tests that allowed me to determine the position of each individual within the shy-bold axis and its association with the expression of melanin-pigmented traits.

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Chapter II

Old males reduce melanin-pigmented traits and increase reproductive outcome under worse environmental conditions in common kestrels*.

David López-Idiáquez, Pablo Vergara, Juan A. Fargallo, Jesús Martínez-Padilla.[1]

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[1] Published Manuscript: López-Idiáquez, D., Vergara, P., Fargallo, J.A. & Martínez-Padilla, J. (2016). Old males reduce melanin-pigmented traits and increase reproductive outcome under worse environmental conditions in common kestrels. *Ecology and Evolution* 6, 1224-1235.

*For consistency, we have used British English in this PhD dissertation, however this chapter is a literal transcription of the paper published in Ecology and Evolution, where American English was used.

ABSTRACT

Secondary sexual traits displayed by males and females may have evolved as a signal of individual quality. However, both individual quality and investment on producing or maintaining enhanced sexual traits change as individuals age. At the same time, the costs associated to produce sexual traits might be attenuated or increased if environmental conditions are benign or worse respectively. Accordingly, environmental conditions are expected to shape the association between the expression of sexual traits and their reproductive outcome as individuals age. Nonetheless, little is known about the environmental influence on the co-variation between sexual traits and reproductive outcome throughout the life of individuals. We studied the age-dependency of the number and size of back spots, a melanin-based and sexual trait in adults of common kestrels (*Falco tinnunculus*). We analysed the age-dependence of reproductive traits and the environmental influence, defined as vole abundance, using a 10-year individual-based dataset. We broke down age-related changes of reproductive traits into within- and between-individual variation to assess their contribution to population-level patterns. Our results showed a within-individual decrease in the number, but not the size, of back spots in males. The size of back spots was positively correlated with food availability in males. Reproductive performance of males increased as they aged, in agreement with the life-history theory but depending of vole abundance. Remarkably, we found that having fewer back spots was positively associated with clutch size only for old individuals under low-food conditions. We suggest that environmental variation may shape the association between the expression of a sexual signal and reproductive outcome. We speculate that the reliability of sexual traits is higher when environmental conditions are poor only for old individuals. Within an evolutionary context, we suggest that the expression of sexual traits might be constrained by environmental conditions at later stages of life.

INTRODUCTION

Sexual selection is the main force driving the evolution and maintenance of secondary sexual traits (Darwin 1871; Andersson 1994). The reliability of these traits depends on the relative cost of production or maintenance, where only high-quality individuals are able to produce and maintain them (Zahavi 1975; Grafen 1990; Kotiaho 2001). However, the expression of secondary sexual traits is not constant over individual lifespan. Senescence, understood as a within-individual deterioration in physiological state over time, may arise because extrinsic mortality rates may reduce the force of selection in late life (Hamilton 1966; Partridge & Barton 1996). Within this life-history context, individuals are expected to increase their investment in sexual signals as their reproductive value decreases to improve mating opportunities in the present (Kokko 1997). However, environmental variation plays a key role in natural and sexual selection, mediating the expression and reliability of sexual traits (Griffith, Owens & Burke 1999; Vergara *et al.* 2012c; a) and the resolution of the trade-off between ornament expression and other functions (Badyaev & Duckworth 2003; Martinez-Padilla *et al.* 2010). Thus, environmental conditions may influence how individuals resolve the trade-off between ornament expression and other costly functions depending on the life-stage of individuals. Scientific literature has focused on different physiological or behavioural mechanisms that explain the variation of ornament expression, but we know rather little about how environmental variation influences the association between the expression of secondary sexual traits and reproductive parameters as individuals age.

In general, knowledge about how the expression of secondary sexual traits (SSTs) changes over the course of an individual's life is scarce, although there has been a recent increase in the number of studies on this topic (Galván & Møller 2009; Forstmeier *et al.* 2006; Torres & Velando 2007; Rivera-Gutierrez, Pinxten & Eens 2010; Velando, Drummond & Torres 2010). Nonetheless, the within- and between-individual effects on age-dependent variation in trait expression has been rarely teased apart (Evans, Gustafsson & Sheldon 2011; Evans & Sheldon 2013; Potti, Canal & Serrano 2013; Potti, Canal & Camacho 2014; Kervinen *et al.* 2015), and this is essential to understand whether trait variation over an individual's life is due to a genuine change in the mean within and among surviving individuals rather than selective disappearance or selective appearance of individuals in the population (Rebke *et al.* 2010). This lack of information is mainly due to the scarcity of long-term and individual-based data collection on the expression of traits in general and SSTs in particular (Nussey *et al.* 2008), raising the possibility that individual life-history trajectories may remain hidden and unknown within the population variation (Bouwhuis *et al.* 2009; Vaupel & Yashin 1985; van de Pol & Wrigth 2009). In spite of the lack of studies disentangling the within- and between-individual age effects in birds, some have tackled the issue from a within-individual perspective (Evans *et al.* 2011; Evans & Sheldon 2013; Potti *et al.* 2013; 2014; Kervinen *et al.* 2015). Still the patterns showed on these studies are uncertain, while some show an increase of ornamentation as individuals age (Evans *et al.* 2011; Evans & Sheldon 2013; Potti *et al.*

2013), others find a lack of senescence on the expression of sexual traits (Candolin 2000a; Miller & Brooks 2005).

The association, however, between ornament expression and reproductive performance throughout the life of individuals is essential to understanding the evolution of sexual traits. According to sexual selection theory, an enhanced expression of sexual traits is expected to be associated with superior reproductive benefits, probably due to prime individual condition or genetic quality of the bearer. This association is maintained because producing or maintaining enhanced expression of sexual traits is traded-off with other energetically demanding functions (Badyaev & Duckworth 2003; Gustafsson, Qvarnström & Sheldon 1995), like reproduction. As a general pattern, individuals at older ages usually reduce their reproductive value (Jones *et al.* 2008), old individuals favouring current mating opportunities, resulting in higher investment in sexual traits (Kokko 1997) as previously reported (Candolin 2000a; b). Thus, the study of age-dependent effects on the expression of sexual traits requires a close examination of its link with reproductive parameters (Evans *et al.* 2011). Only two studies have explored the covariation between within-individual age variation of sexual traits and reproduction in birds. In collared flycatchers (*Ficedula albicollis*), only females decreased their reproductive output while increasing the expression of their ornament as they aged (Evans *et al.* 2011). In pied flycatchers (*Ficedula hypoleuca*), females only decreased their reproductive output, though increased the expression of the trait, late in life if they expressed a forehead patch at early ages (first breeding attempt – Potti *et al.* 2013). Overall, these studies suggest that ornament expression with respect to age might be traded-off with other resources, particularly with those allocated to reproduction. However, the sex-dependent effect and few examples regarding within-age expression of ornamental traits indicate a need for caution in generating accurate predictions within a life-history context.

Crucial, however, is the notion that environmental conditions may change the intensity of sexual selection. In birds, examples of how the environment mediates the expression of sexual traits (Griffith *et al.* 1999; Chaine & Lyon 2008; Martinez-Padilla *et al.* 2010; Robinson *et al.* 2012; Vergara & Martínez-Padilla 2012; Vergara *et al.* 2012b; c; Martínez-Padilla *et al.* 2014), the condition-dependence of their expression (Vergara *et al.* 2012a) and allocation priorities (Martinez-Padilla *et al.* 2010; Vergara & Martínez-Padilla 2012; Martínez-Padilla *et al.* 2014) are well-known. From the recent scientific literature, it can be assumed that worse environmental conditions reduce the expression of ornamental traits (Cotton, Fowler & Pomiankowski 2004), and increase the reliability of sexual traits (Vergara *et al.* 2009; 2012a). Thus, only high-quality individuals are able to express enhanced traits and reliably inform about individual quality under worse environmental conditions. Within a life-history perspective, environmental conditions might also shape the abovementioned trade-off between the investment in sexual traits and reproduction. Specifically, if environmental conditions are poor, individuals at older ages are expected to increase the investment in current mating opportunities and reproduction due to their reduced reproductive value. Otherwise, if environmental conditions are benign, the investment in producing an enhanced sexual trait and breeding

outcome might be affordable for all individuals regardless of their age because resource abundance may allow them to confront these two energetically demanding functions.

Here, we aim to study within-individual and lifetime variation of secondary sexual traits in both adult breeding male and female common kestrels (*Falco tinnunculus*). Specifically, we explored how age covaries with reproductive parameters, and how environmental variation modulates this association in both males and females. We measured the number and size of black back spots in kestrel plumage, a trait that plays a role in sexual selection (Palokangas *et al.* 1994). Black coloration in kestrel plumage has been described to be based on melanin pigments (Fargallo *et al.* 2007a; b), showing both environmental and genetic components in its expression (Vergara *et al.* 2009; Kim *et al.* 2013). To explore the environmental influence, we used the abundance of common voles (*Microtus arvalis*) as a proxy of environmental variation, since the abundance of breeding kestrels in our population is sensitive to the interannual fluctuation of vole density (Fargallo *et al.* 2009). As a general prediction, we expect a stronger covariation between the expression of sexual traits and reproduction at older ages when environmental conditions are worse. We base our general prediction in three subsequent expectations that will be also explored: (1) within-individual variation in the number and size of spots as individuals age; (2) covariation between ornamentation and reproductive performance; and (3) the association between environmental variation and ornament expression.

MATERIAL AND METHODS

Study species

The common kestrel (hereafter kestrel) is a medium-sized raptor that exhibits marked sexual dimorphism in body size (females are 20% heavier than males) and in plumage coloration (Village 1990; Palokangas *et al.* 1994). Adult males range from brownish-red to brick red with black spots on their backs and on the upper sides of their wings. Their heads range from completely brown to completely grey, and their rumps, upper tail-coverts and tails are grey and mainly unbarred. Females are brown on the head, back and upperside of the wings, always with black spots. Juveniles show variable moulting of body feathers during their first year of life while maintaining most of their plumage during their first breeding season. Males and females differ in the expression of their back spots, with males showing less spots, mainly in adulthood (age ≥ 2). Back spots in males has been proved to have a role in sexual selection in kestrels (Palokangas *et al.* 1994). In barn owls (*Tyto alba*), females show a similar pattern of spottiness of a melanin-pigmented trait (Roulin 2004), being a sexually selected trait that reflects genetic quality (Roulin 2004) and influence male mate choice (Roulin 1999), suggesting that spottiness might be sexually selected in females of our study species. Kestrel moult takes place usually after breeding (Village 1990). Because previous studies show that yearlings have a delayed plumage maturation (Village 1990; Vergara & Fargallo 2007), we only analysed adults (age ≥ 2) to avoid first-year plumage (Evans *et al.* 2011).

Study area

The study was conducted in the Campo Azálvaro region (40º 40' N, 4º 20' W), a homogeneous mountain grassland area in central Spain (1300 meters a.s.l) devoted mainly to cattle-raising. Nest-boxes were installed in the area progressively from 1994 to 2005, and the breeding population has been followed since then (Fargallo *et al.* 2001). During the period in which the study took place (2004– 2013), there were a range of 24–45 kestrel pairs breeding each year (Fargallo *et al.* 2009).

Data collection

This study took place between the breeding seasons of 2004 and 2013. Nests were monitored to detect laying date (the day that the first egg was laid), clutch size (mean = 5, range = 3–7, n = 277), and number of fledglings (mean = 4, range = 1–7, n = 277). Adult breeders were captured when nestlings were 10–13 days old (n = 277; males: n = 143; females: n = 134). At that time, body mass (to the nearest g), wing and tarsus length (to the nearest mm) were recorded, and a digital photograph was taken (see Supporting Information - Nikon D70; objective: 18–70 mm AF-S Nikkor DX). All photographs included the back and the right wing of every individual, along a scale that allowed us to determine the size of each trait in mm^2. We determined whether they were yearlings (1-year-old) or adults (≥2 years old) by using ring codes or plumage features (Vergara & Fargallo 2007).

Ornament assessment

We measured the number and size of back spots in different areas of the wing and back of adult male and female kestrels using the photographs taken during their capture. The size of the back spots was the mean size value of five randomly selected spots using the "loop" tool of Photoshop CS5. Number and size of spots differed in their correlational level with the number and size of the spots in the other areas of the kestrel (see SM-2.1 Tables from SM 2.1 to SM 2.4 for further details) and they were not intercorrelated, either in males or in females (see SM-1 Tables SM 2.5 and SM 2.6 for further details). We also analysed the repeatability (Lessells & Boag 1987) of our spot size measure finding highly repeatable measurements only in the back spots (males r = 0.74; females r = 0.71; see SM-2.2 Table SM 2.7 for further details). Thus, we decided to use only the number and size of back spots in the subsequent analyses.

Environmental variables

The abundance of common voles was assessed by two trapping sessions per year. These were carried out in autumn and spring by setting out one hundred Sherman traps in four plots (25 each) for the length of the study. All traps were monitored six times for 3 days, three times in the early morning and three at sunset, during new moon periods to avoid the effects of moonlight on small mammal activity (Fargallo *et al.* 2009).

Statistics and modelling

All analyses were carried out in R statistical software (packages "*lme4*" and "*lmerTest*" - Bates, Maechler & Bolker 2013; Kuznetsova, Brockhoff & Christensen 2013). Kestrels show a remarkable sexual dimorphism in plumage (Village 1990), with males and females showing significant differences in both number ($F_{1,178}$ = 312.5 P < 0.001) and size ($F_{1,178}$= 47.42, P < 0.001) of back spots. Thus, we analysed males and females separately in all models described below. In all statistical models, we followed a backwards-stepwise selection procedure, in which all terms were initially included. Non-significant terms (P > 0.05) were removed sequentially.

Melanin-based traits as proxies of individual quality

To establish the relationship between the ornamental traits and individual quality we performed Linear Mixed Models (LMMs). We first analysed the association between number and size of spots (dependent variables) and body mass or wing length in different models. Secondly, we explored the association between three reproductive variables (laying date, clutch size, and number of fledglings) and number and size of spots in different models. In this case, number and size of spots were included as explanatory variables and reproductive variables as dependent variables. In all models, age was included as a covariate, and individual identity and nest as random factors.

Age-specific traits expression and reproductive performance

We first explored the cross-sectional relationship between ornament expression and age. All colour traits were z-transformed (to mean = 0 and standard deviation = 1; z-scores) to aid direct comparison across traits for each sex separately. We constructed Linear Mixed Models (LMM) with individual identity as a random factor. Age was fitted as a linear as well as quadratic effect and year as covariate (Conover & Schultz 1995; Conover, Duffy & Hice 2009).

Secondly, in order to explore within-individual variation (WIA) on the expression of size and number of back spots, we developed longitudinal analyses, using a within-subject centering approach to partition the population level age variation into within- and between-individual effects (van de Pol & Wrigth 2009; Dingemanse & Dochtermann 2013). The within-individual term is calculated by subtracting an individual's mean age from each individual age value (Within-individual age – WIA= x_{ij} - x_j, where x_{ij} is the age value of individual j at year i, and x_j is the mean age of individual j in the dataset; van de Pol & Wrigth 2009). We also considered age at the last measurement (ALM) in order to take into account the effects of selective disappearance caused by an age-mediated mortality pattern of individuals (van de Pol & Verhulst 2006). In order to control for the selective appearance of immigrant individuals coming from other populations with a different phenotypic expression, we considered the age of the first reproductive event of individuals in our population (AFM; van de Pol & Verhulst 2006). We constructed LMMs, considering number and size of spots (dependent variables) and ALM, AFM, and

WIA as covariates. Individual identity was considered as a random factor.

We constructed LMMs with reproductive traits as dependent variables (laying date, clutch size, and number of fledglings) to explore the covariation between reproductive traits and ornamental traits. Lack of sample size did not allow us to explore the covariation between ornament expression and reproductive parameters in relation to age and environmental variation from a reaction norm perspective. To control for the possible effects of year in our models, ornamental and reproductive traits were z-transformed for each year to aid direct comparison (see above). WIA, ALM, and AFM were included as independent variables. LMM models were fitted to a normal distribution of errors and individual identity as a random factor. We also included the interactions between the independent values. We included breeding seasons from 2004 to 2012 in the analyses, using the 2013 data to improve the accuracy of the ALM estimates.

Environmental variation

In order to assess the environmental influence on melanin- based traits we used the number of voles in the previous spring (NVPS) capturing the food conditions that adult kestrels experienced at the time of moulting. We conducted LMMs for the number and size of spots as dependent variables and NVPS as explicative, individual identity was fitted as a random effect and individual age as a covariate. This analysis has a cross-sectional perspective and does not allow us to tease apart the within- from the between-individual effects of NVPS. From a longitudinal perspective, we grouped NVPS into five categories, according to the quartiles ($NVPS_q$). We then used these categories to calculate a new variable (Within-individual "Vole"- WIV= x_{ij}-x_j, where x_{ij} is the $NVPS_q$ value of individual j at year i, and x_j in the mean $NVPS_q$ value of individual j in the dataset), analogous to WIA, that allowed us to tease apart the within-individual effects of the environment. We included this variable as explicative in order to explain the variation in the number and size of back spots.

In order to explore the effect of environmental variation on the relationship between age and reproduction, we categorized NVPS into high and low if annual values were above or below the overall mean ($NVPS_c$, coded as "high" or "low"). Due to sample size limitations, we also categorized WIA (WIA_c) of each individual in three levels as -1, 0 and 1, representing early ages (-1: $WIA_c < 0$), midlife ages (0: $WIA_c = 0$), and late life (1: $WIA_c > 0$). Melanin-based traits were z-transformed for the categories of $NVSP_c$ and WIA_c. $NVPS_c$, WIA_c and the z-scores of the number and size of spots were the explanatory variables in our models. Clutch size and number of offspring were considered proxies of fitness and were mean-centered for each category of $NVSP_c$ and WIA_c, and included as dependent variables in our models. We tested the interaction between $NVPS_c$, WIA_c and ornament expression in explaining variation of clutch size or number of offspring. The models included year and individual identity as random effects and were carried out separately for the number and size of spots and for males and females.

RESULTS

Melanin-based traits as proxies of individual quality

We did not find any association between wing length on the expression of the studied melanin-based traits, either for males ($P > 0.153$) or for females ($P > 0.208$). However, we found that spot size was positively correlated to body mass in females (0.082 ± 0.038, $F_{1,57.86} = 4.47$, $P = 0.039$) but not in males ($F_{1,97.59} = 0.00007$, $P = 0.993$). No significant relationship was found between spot number and body mass, either in females ($F_{1,58.9} = 0.45$, $P = 0.505$) or in males ($F_{1,95.28} = 0.176$, $P = 0.675$).

In males, there was a significant negative relationship between spot size and laying date (estimate = -0.474 ± 0.202, $F_{1,90.09} = 5.469$, $P = 0.021$). However, spot size was not correlated with clutch size ($F_{1,97.65} = 7.936$, $P = 0.407$) and marginally positively with number of fledglings (0.058 ± 0.030 $F_{1,88.49} = 3.727$, $P = 0.056$). No significant relationship was found between number of spots and the reproductive variables ($P > 0.408$). In females, our results show that laying date was positively correlated with number of spots (0.148 ± 0.067, $F_{1,51.04} = 4.783$, $P = 0.033$) and negatively with size of spots (-0.470 ± 0.195, $F_{1,60.59} = 5.807$, $P = 0.019$). No significant relationships were found for clutch size or number of fledglings neither with spot number (CS: $F_{1,67.09} = 0.042$, $P = 0.837$, NF: $F_{1,53.97} = 0.271$, $P = 0.604$) or spot size (CS: $F_{1,69.72} = 0.242$, $P = 0.624$, NF: $F_{1,72.1} = 2.849$, $P = 0.095$).

Cross-sectional patterns of ornamental trait change

The cross-sectional analyses showed a significant negative relationship between age and number of back spots in males but not in females (Table 2.1). We also found that age was not a significant predictor of the size of back spots, either in males or in females (Table 1). Year was retained as a significant negative predictor for the number of spots in males (Table 2.1).

Within-individual patterns of ornamental and reproductive change

We found that WIA was a significant predictor of the number back spots in males (Table 2.2, Fig. 1). No significant relationship was found for the size of back spots in males or females (Table 2.2). Regarding reproductive performance in males, we found that the number of fledglings was positively correlated with ALM and marginally with WIA (Table 2.3). We also found a significant relationship between laying date and ALM (Table 2.3). Finally, we found that WIA and AFM, and their interaction were significant and positive predictors of clutch size. In females, we found no significant relationship with any of the studied variables (Tables 2.2 and 2.3).

Parameter	Estimate	SE	*F*	*P*	*E. Seq.*
Male number of back spots (n=105)					
Age	**-0.156**	**0.06**	**$F_{1,32}$=5.354**	**0.027**	
Age^2	-0.006	0.04	$F_{1,31}$=0.023	0.879	1
Year	**-0.129**	**0.03**	**$F_{1,32}$=10.92**	**0.002**	
Male size of back spots (n=105)					
Age	0.008	0.07	$F_{1,33}$=0.012	0.911	
Age^2	-0.059	0.04	$F_{1,32}$=1.631	0.210	2
Year	0.026	0.04	$F_{1,31}$=0.335	0.566	1
Female number of back spots (n=75)					
Age	0.028	0.06	$F_{1,30}$=0.193	0.663	
Age^2	-0.030	0.03	$F_{1,29}$=1.001	0.325	2
Year	0.014	0.05	$F_{1,28}$=0.021	0.787	1
Female size of back spots (n=75)					
Age	-0.014	0.06	$F_{1,30}$=0.045	0.832	
Age^2	-0.022	0.03	$F_{1,29}$=0.537	0.469	2
Year	0.029	0.05	$F_{1,28}$=0.223	0.594	1

Table 2.1: Results of cross-sectional models of expression of melanin-based traits in male and female kestrels aged ≥ 2. Variables included in the final model are in bold; values for the excluded variables refer to the step before their exclusion (E. Seq.).

Parameter	Estimate	SE	*F*	*P*	*E. Seq.*
Male number of back spots (n=105)					
WIA	**-0.289**	**0.111**	**$F_{1,33}$=6.803**	**0.013**	
AFM	-0.106	0.113	$F_{1,68}$=0.708	0.348	3
ALM	-0.074	0.072	$F_{1,69}$=1.076	0.303	5
WIA*AFM	0.059	0.185	$F_{1,31}$=0.060	0.798	1
WIA*ALM	-0.089	0.086	$F_{1,32}$=1.077	0.307	4
AFM*ALM	0.035	0.070	$F_{1,67}$=0.252	0.616	2
Male size of back spots (n=105)					
WIA	-0.091	0.115	$F_{1,33}$=0.476	0.433	5
AFM	-0.072	0.109	$F_{1,68}$=0.023	0.531	3
ALM	0.070	0.071	$F_{1,69}$=0.966	0.329	
WIA*AFM	-0.020	0.198	$F_{1,31}$=0.092	0.919	1
WIA*ALM	-0.115	0.087	$F_{1,32}$=1.747	0.195	4
AFM*ALM	-0.027	0.069	$F_{1,67}$=0.154	0.695	2
Female number of back spots (n=75)					
WIA	-0.051	0.121	$F_{1,30}$=0.026	0.675	5
AFM	-0.133	0.184	$F_{1,41}$=0.745	0.475	3
ALM	0.039	0.054	$F_{1,42}$=0.535	0.648	
WIA*AFM	-0.034	0.201	$F_{1,28}$=0.693	0.863	2
WIA*ALM	-0.100	0.056	$F_{1,29}$=3.181	0.085	4
AFM*ALM	0.018	0.113	$F_{1,40}$=0.027	0.869	1
Female size of back spots (n=75)					
WIA	0.007	0.110	$F_{1,30}$=0.002	0.947	5
AFM	0.022	0.180	$F_{1,42}$=0.015	0.901	
ALM	-0.025	0.076	$F_{1,41}$=0.150	0.739	3
WIA*AFM	-0.243	0.164	$F_{1,29}$=2.197	0.149	4
WIA*ALM	0.053	0.063	$F_{1,28}$=0.002	0.412	1
AFM*ALM	0.110	0.120	$F_{1,40}$=0.842	0.364	2

Table 2.2: Results of individual-level models of expression of age-dependent plumage melanin in male and female kestrels of known age (≥2). The annual mean expression level was subtracted from each trait measurement to control for population-level variation in ornamentation, before standardization (see M&M for further details). Within-individual age (WIA) represents the individual change during the life of a given individual. Age at measurement (ALM) is a proxy of longevity. Age at first measurement (AFM) represents the first reproduction in our population. Variables included in the final model are in bold. Variables included in the final model are in bold; values for the excluded variables refer to the step before their exclusion (E. Seq.).

Association between environmental variation and ornamental traits

The cross-sectional approach showed a significant and positive relationship between $NVPS_q$ and the size of male back spots (estimate: 0.011 ± 0.004; $F_{1,33} = 6.353$ P = 0.016 – Fig. 2.2). We then analysed the relationship between the within-individual effect of voles (WIV) and the melanin-based traits. We found a positive association between the WIV and the size of male back spots (estimate: 0.198 ± 0.091; $F_{1,33} = 4.761$ P = 0.036). No other significant relationships were found in males or females (P > 0.079).

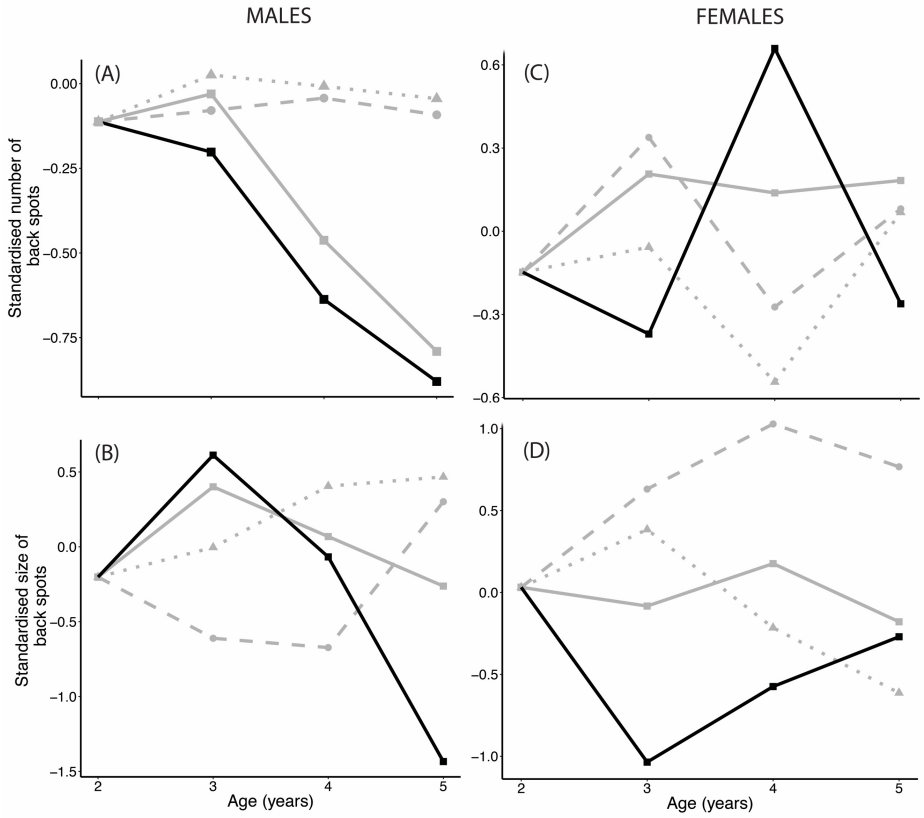

Figure 2.1: Patterns of standardized melanin- based trait expression for males (A, B) and females (C, D) of known age (≥2 years). The thick black line with squares represents within- individual change. The thick grey line with squares represents the population level pattern. The dashed light grey with circles line shows the contribution of the selective appearance and the spotted grey line with triangles shows the contribution of the selective disappearance to the population-level mean. Note that the scale may differ between graphs. To perform these graphics, we followed Rebke et al. (2010). We only represented age of birds up to 5 years old because of the limited sample size of individuals at higher ages classes (Age 6 = 4, 7 = 1 and 8 = 1; see SM-2.3 Table SM 2.8 for further details).

Parameter	Estimate	SE	*F*	*P*	*E. Seq.*	Estimate	SE	*F*	*P*	*E. Seq.*
	(Male) Number of fledglings (n=109)					(Female) Number of fledglings (n=75)				
WIA	0.297	0.153	$F_{1,35}$=4.486	0.061		0.011	0.116	$F_{1,30}$=0.00002	0.921	5
AFM	0.082	0.116	$F_{1,70}$=3.469	0.481	4	0.097	0.163	$F_{1,42}$=0.535	0.555	
ALM	**0.189**	**0.075**	**$F_{1,71}$=6.27**	**0.014**		-0.006	0.062	$F_{1,41}$=0.051	0.912	3
WIA*AFM	-0.287	0.236	$F_{1,34}$=0.502	0.282	2	-0.263	0.178	$F_{1,29}$=2.182	0.150	4
WIA*ALM	-0.011	0.113	$F_{1,33}$=0.157	0.918	1	0.021	0.068	$F_{1,28}$=0.103	0.750	2
AFM*ALM	-0.129	0.076	$F_{1,69}$=2.918	0.092	3	0.0005	0.113	$F_{1,40}$=0.00002	0.996	1
	(Male) Laying date (n=109)					(Female) Laying date (n=75)				
WIA	-0.090	0.131	$F_{1,35}$=0.533	0.497	5	-0.146	0.109	$F_{1,30}$=0.524	0.190	4
AFM	-0.100	0.100	$F_{1,70}$=3.469	0.481	3	-0.241	0.187	$F_{1,41}$=0.917	0.204	5
ALM	**-0.146**	**0.064**	**$F_{1,71}$=5.13**	**0.026**		0.069	0.066	$F_{1,42}$=1.115	0.297	
WIA*AFM	0.187	0.218	$F_{1,33}$=0.367	0.379	2	0.195	0.156	$F_{1,29}$=1.563	0.221	3
WIA*ALM	-0.106	0.090	$F_{1,34}$=1.369	0.250	4	-0.085	0.059	$F_{1,28}$=0.565	0.158	2
AFM*ALM	-0.0007	0.069	$F_{1,69}$=0.000	0.991	1	0.059	0.118	$F_{1,40}$=0.246	0.622	1
	(Male) Clutch size (n=109)					(Female) Clutch size (n=75)				
WIA	**0.975**	**0.352**	**$F_{1,34}$=4.61**	**0.009**		-0.139	0.109	$F_{1,30}$=1.622	0.212	
AFM	**0.202**	**0.088**	**$F_{1,71}$=4.16**	**0.024**		0.101	0.172	$F_{1,42}$=0.344	0.560	5
ALM	0.083	0.087	$F_{1,70}$=1.044	0.342	3	0.007	0.067	$F_{1,41}$=0.0009	0.907	3
WIA*AFM	**-0.371**	**0.169**	**$F_{1,34}$=4.77**	**0.035**		-0.317	0.166	$F_{1,29}$=3.630	0.066	4
WIA*ALM	0.037	0.086	$F_{1,33}$=0.343	0.670	1	-0.033	0.064	$F_{1,28}$=0.273	0.605	2
AFM*ALM	-0.045	0.065	$F_{1,69}$=0.471	0.494	2	-0.053	0.113	$F_{1,40}$=0.236	0.629	1

Table 2.3: Results of individual-level models of the reproductive traits in male and female kestrels of known age (≥2). The annual mean expression level was subtracted from each trait measurement to control for population-wide variation in ornamentation, before standardization by z-transformation. Variables included in the final model are in bold. Variables included in the final model are in bold; values for the excluded variables refer to the step before their exclusion (E. Seq.).

Environmental influence on the association between reproduction and ornament expression

In males, we found that the association between clutch size and number of back spots was mediated by WIA_c and $NVPS_c$ (interaction number of back spots*WIA_c*$NVPS_c$; $F_{2,82.337}$ = 3.949, P = 0.023). We split this triple interaction and explored the relationship between clutch size and number of spots under the high and low food availability scenarios. In a low food scenario, we found that the significant relationship between clutch size and number of spots was mediated by WIA_c (interaction number of back spots*WIA_c; $F_{2,45.45}$ = 3.315; P = 0.045; Fig. 2.3). In particular, we found a negative association between clutch size and number of back spots only in individuals at later stages of life ($WIA_c > 0$ estimate = 0.098 ± 0.033, $F_{1,21}$ = 8.796 P=0.007; P>0.582 for WIA_c =0 and WIA_c <0; Fig. 2.3). Under a high food availability context, our results also show a significant influence of WIA_c in the relationship between the clutch size and the number of spots ($F_{2,5.014}$ = 10.563, P = 0.015). Specifically, we found a significant association between clutch size and number of back spots only in individuals at midlife (WIA_c = 0: 0.049 ± 0.019 $F_{1,22}$ = 6.221 P = 0.020). We also found a marginal tendency for early life individuals (WIA_c < 0: -0.087 ± 0.042, $F_{1,10}$ = 4.204, P = 0.067) and no significant relationship for those in age class 3 ($WIA_c > 0$ P = 0.344). Number of fledglings and number of back spots were not mediated by WIA_c and $NVPS_c$ (interaction number of back spots*WIA_c*$NVPS_c$; $F_{2,93}$ = 0.398, P = 0.692). Regarding size of back spots, we did not find any mediating effect of WIA_c or $NVPS_c$ on any proxy of fitness (clutch size: interaction size of back spots*WIA_c*$NVPS_c$; $F_{2,83.163}$ = 2.943, P = 0.058; number of fledglings: number of back spots*WIA_c*$NVPS_c$; $F_{2,92.356}$ = 2.686, P = 0.073). We did not find any relationship in females (P > 0.177).

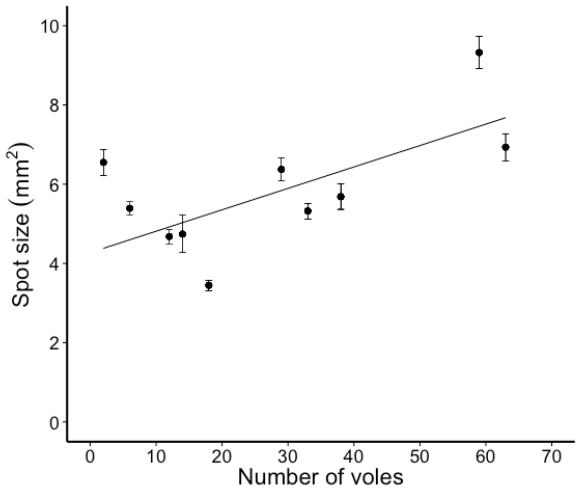

Figure 2.2: Positive cross-sectional pattern of vole abundance of previous spring (NVPS) on back spot size (mean ± SD) of male kestrels of known age (≥2 years).

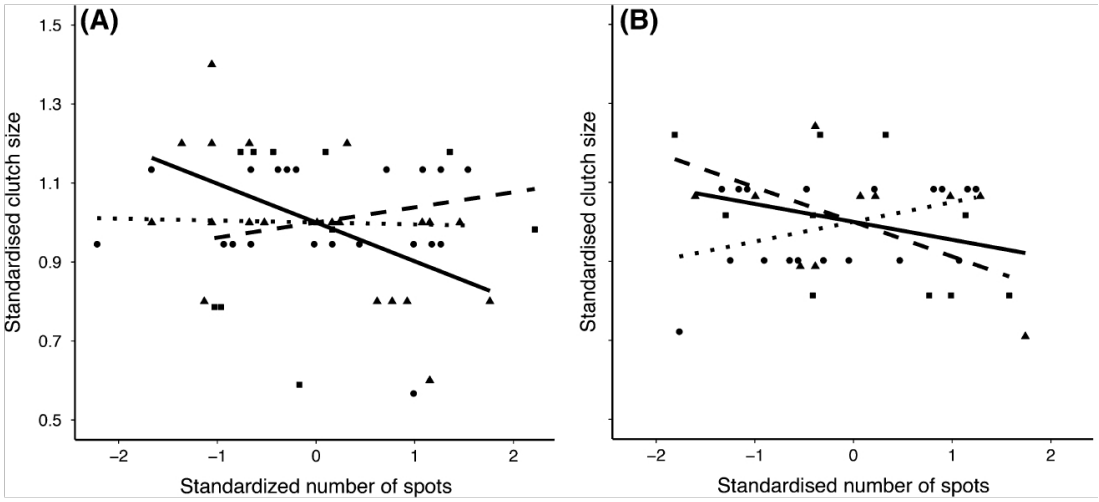

Figure 2.3: Relationship between clutch size and number of spots of known age of males of common kestrel under low (A) and high (B) vole abundance. The lines represent individuals at three different stages of life: Early life (short dashed line and black squares), mid-life (dotted line and black circles), and late life (black line and black triangles). See methods for further details.

DISCUSSION

We used a 10-year dataset to investigate the influence of age and environmental variation on the expression of ornamental melanin-based traits and compared it with aging patterns of reproductive characters. Cross-sectional and within-individual analyses show that number, but not size, of spots decreases as individuals age, only in males. This suggests that the number of spots may act as an index of quality as individuals that live longer show less spots, providing a proxy of quality and perhaps fitness benefits for females (Kokko & Lindstrom 1996; Brooks & Kemp 2001; Kokko 1998). This is partially supported by our results, where we found that older males produce larger clutch sizes and raise more offspring. In females, back spots may be a reliable index of individual quality, since females showing more and larger spots had earlier laying dates.

Interestingly, we found an increase in reproductive performance with age in males, as predicted by life-history theory (Williams 1966). Specifically, clutch size increases as males age and is larger for individuals whose first breeding event is earlier in their lives. In addition, brood size also increases as individuals age and is larger for individuals that live longer. This is consistent with the idea that by mating with older males, females may obtain more fitness benefits than by mating with younger ones (Kokko & Lindstrom 1996; Brooks & Kemp 2001; Kokko 1998). Regarding the association between reproductive output and number of spots, our results fit within a life-history context and disagree with the senescence-based scenario, which predicts a decrease in reproductive performance throughout life. We cannot rule out the possibility that individuals may show such senescence patterns in reproduction at older ages, undetected here either because of a deficit in the number of older individuals or a stronger selection in this species against senescent individuals.

Our results suggest that environmental circumstances may drive the association between trait expression and fitness, considering clutch size as a proxy, at different life stages. Our results reveal that number of spots and clutch size are negatively correlated only for individuals in later periods of life under a scenario of low food availability. Considering the association between fitness and number of back spots as an individual index of quality, females will obtain reproductive benefits when choosing older (less spotted) individuals as mates. But this benefit will increase under worse environmental conditions, which is in our case low food abundance. Under this scenario and supporting our general prediction, the reliability of the signal increases when environmental conditions worsen and particularly for old individuals. This may explain why individuals decrease their number of back spots at older ages. Within an evolutionary context, it is possible that environmental fluctuation may prevent a change in the frequency distribution of the number of back spots in males in our population since high or low food quality scenarios fluctuate, although not cyclically, favouring fluctuating selection. These results suggest that environmental conditions may shape the strength of selection on ornamental traits during different stages of an individual's life. Unfortunately, specific analyses studying the selection of the covariation between ornamental traits and reproduction in relation to aging and environmental conditions are needed to confirm this pattern. Our results also show that under benign environmental conditions middle-aged individuals with high number of spots have larger clutch sizes. These results may explain the variance in the expression of number of spots in different individuals, as they may gain mating benefits depending on the environmental conditions.

It is common in the study of the function or evolutionary dynamics of sexual traits to consider the size or coloration of given traits, but little is known when exploring the expression of different characteristics of the same trait. We found that number but not size of back spots changes throughout life and that size but not number is influenced by food availability. The size of back spots changes in accordance to the environmental situations that individuals face during their lives. A key environmental feature that explains the size of spots in male breeding kestrels appears to be the abundance of common voles, a key prey for kestrels in our population (Navarro-López, Vergara & Fargallo 2014). This result agrees with the idea of environmental influence mediating the expression of melanin-based traits (Fargallo *et al.* 2007a; b; Vergara *et al.* 2009) even when they have a strong genetic influence in kestrels (Kim *et al.* 2013). We suggest that the size of back spots may provide information about male food intake during moult, as they may influence the hormonal production that regulates melanogenesis (Jawor & Breitwisch 2003).

In spite of previous studies showing that melanin-based coloration is under genetic control (Roulin & Dijkstra 2003; Niecke, Rothlaender & Roulin 2003; Griffith, Parker & Olson 2006; Kim *et al.* 2013), the role of environmental variance in the expression of melanin-based traits is striking (Fargallo *et al.* 2007b; Vergara *et al.* 2009; Kim *et al.* 2013). Our results suggest that the size of back spots might be a reliable index of a male's ability to obtain high-quality territories in terms of food availability, while the expression

of the number of back spots might be more associated with non-environmental sources of variation. Therefore, two characteristics of the same melanin-based trait can be driven by different factors. Perhaps additive genetic variance explains a higher proportion of total variance of the expression of number of spots in males, while food abundance drives the size of back spots. Regardless of the specific factors explaining the variance of the expression of these two characteristics, it seems plausible to think that these two characteristics may work in a multiple message context, indicating the different characteristics of individuals (Candolin 2003; Møller & Pomiankowski 1993; Chaine & Lyon 2008). On the one hand, the number of spots may convey information about the genetic quality of the individuals, and on the other hand, the size of back spots may provide information about males' skills for obtaining good territories during moult.

Our study also reveals a sex-dependent variation in the expression of back spots and reproductive output. Reasons for such variation are uncertain but raise a key question in associating age-dependent variation of melanin-based traits with sexual dimorphism. Females change the expression of melanin-based traits very little throughout their lives in comparison to males. Specifically, males do change their phenotype showing a less young-like phenotype (less spots), increasing sexual dimorphism as individuals age. This agrees with our results where we found a positive selection on more experienced males with fewer back spots, an effect that becomes stronger in worse environ- mental (low food) conditions. A potential covariation between an age-dependent expression of melanic traits and reproduction in males, but not in females, may potentially provide new venues for the study of the evolution of sexual dimorphism in the expression of melanin-pigmented traits.

Overall, our study suggests a sex-dependent effect of melanin-based back coloration, where there are within-individual decreases with age in the number of back spots only in males. Within-age decreases in the number of back spots and the increase in reproductive performance in males as they age is in agreement with a life-history perspective but not so with the senescence context. Finally, our results point toward an environment-mediated selection on the expression of phenotypes particularly stronger in later stages of life. We suggest that environmentally and genetically driven selection forces may act differentially on two characteristics of the same melanin-based trait, explaining the expression of the same trait.

ACKNOWLEDGEMENTS

The authors dedicate this paper to the memory of Pablo Vergara, who passed away in August 2013 during the field- work and analyses of this manuscript. We thank J. Navarro-López and I. López-Rull for their help during fieldwork. The study was carried out on the Finat family property with the help of J. San Teodoro. Licenses to study kestrels were granted by the Junta of Castilla y León. The long-term monitoring of the study population was financed by several projects of the Ministerio de Economía y Competitividad (CGL2004-04479/BOS, CGL2007-61395/ BOS, CGL2010-15726/BOS, CGL2013-42451-P) of the Spanish Government. This is a contribution of the El Ventorrillo Biological Station.

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SUPPLEMENTARY MATERIAL

SM-2.1: Inter-relationship melanin-based measured traits

Preliminarily we analysed the inter-relationship between the common kestrel's plumage spots. With this aim we divided kestrel's dorsum in four areas (a) back, (b) auxiliary feathers, (c) greater coverts and (d) median and lesser coverts (Fig. SM 2.1). In each of them we counted the number of spots and randomly selected 5 of them to measure the size, except for the great coverts where we always measured the same spot (red circle). Then we constructed LMM, between the number of spots and the size in each area for both males and females. We included individual identity as a random effect to avoid pseudoreplication (Hurlbert 1984) because we have repeated measures of the same individuals. Our results show that there is a high positive relation between the number of spots (Table SM 2.1) and the area in the four zones of male's kestrel dorsum (Table SM 2.2). That is, if one male has a great number of spots in the back, it will have a lot of spots in the other patches and the same with the back-spot size. In the case of the females we did not found any consistent pattern (Tables SM 2.3 and SM 2.4). We also analysed the relation between the number and size of the spots in each patch. For both males and females (Tables SM 2.6 and SM 2.7) there is not a consistent pattern in the relationship suggesting that these two variables have different influences.

	Nº of back spots	Nº of auxiliary feather spots	Nº of greater covert spots	Nº of median and lesser covers spots
Nº of back spots		1.201±0.176****	0.658±0.234***	0.249±0.032****
Nº of auxiliary feather spots	1.201±0.176****		0.167±0.088*	0.087±0.012****
Nº of greater covert spots	0.658±0.234***	0.167±0.088*		0.041±0.011***
Nº of median and lesser covers spots	0.249±0.032****	0.087±0.012****	0.041±0.011***	

Table SM 2.1: Results of LMM analysing the inter-relationship of the spot number in four patches of male common kestrels (*Falco tinnunculus*). *P<0.1 (Marginally significant) **P<0.05 ***P<0.01****P<0.0001 (n=175).

	Back spot size	Auxiliary feather spots size	Greater covert spot size	Median and lesser covers spot size
Back spot size		0.254±0.038****	0.050±0.138***	0.352±0.101****
Auxiliary feather spots size	0.254±0.038****		0.106±0.024***	0.762±0.176***
Greater covert spot size	0.050±0.138***	0.106±0.024***		1.917±0.532***
Median and lesser covers spot	0.352±0.101****	0.762±0.176***	1.917±0.532***	

Table SM 2.2: Results of LMM with normal errors analysing the inter-relationship of the spot size in four patches of male common kestrels (*Falco tinnunculus*). Significant variables are in bold. *P<0.1 (Marginally significant) **P<0.05 ***P<0.01****P<0.0001 (n=175).

	Nº of back spots	Nº of auxiliary feather spots	Nº of greater covert spots	Nº of median and lesser covers spots
Nº of back spots		0.379±0.182	0.002±0.341	0.099±0.080
Nº of auxiliary feather spots	0.379±0.182		0.0533±0.202**	0.141±0.050**
Nº of greater covert spots	0.002±0.341	0.0533±0.202**		0.034±0.028
Nº of median and lesser covers spots	0.099±0.080	0.141±0.050**	0.034±0.028	

Table SM 2.3: Results of LMM analysing the inter-relationship of the spot number in four patches of female common kestrels (*Falco tinnunculus*). *P<0.1 (Marginally significant) **P<0.05 ***P<0.01****P<0.0001 (n=68).

	Back spot size	Auxiliary feather spots size	Greater covert spot size	Median and lesser covers spot size
Back spot size		0.076±0.073	0.012±0.034	0.163±0.133
Auxiliary feather spots size	0.076±0.073		0.065±0.058	0.456±0.209**
Greater covert spot size	0.012±0.034	0.065±0.058		1.058±0.392**
Median and lesser covers spot size	0.163±0.133	0.456±0.209**	1.058±0.392**	

Table SM 2.4: Results of GLMM with normal errors analysing the inter-relationship of the spot size in four patches of female common kestrels (*Falco tinnunculus*). *P<0.1 (Marginally significant) **P<0.05 ***P<0.01****P<0.0001 (n=68).

Parameter	Estimate	SE	F	P
Number of back spots (n=175)				
Back spot size	0.146	0.263	$F_{1,49}$=0.311	0.579
Number of auxiliary feathers spots (n=175)				
Auxiliary feather spot size	0.069	0.055	$F_{1,49}$=1.566	0.216
Number of greater coverts spots (n=175)				
Size of greater covert spots	**0.054**	**0.015**	**$F_{1,49}$=13.258**	**0.0007**
Number of median and lesser coverts (n=175)				
Size of median and lesser covert spots	0.152	0.716	$F_{1,49}$=0.045	0.831

Table SM 2.5: Results of the LMM analysing the relationship between the number and size of the spots in each patch in male common kestrels (*Falco tinnunculus*). Significant variables are in bold.

Parameter	Estimate	SE	*F*	*P*
Number of back spots (n=68)				
Back spot size	-0.088	0.337	$F_{1,19}$=0.068	0.795
Number of auxiliary feather spots (n=68)				
Auxiliary feather spot size	**-0.441**	**0.122**	**$F_{1,19}$=13.055**	**0.001**
Number of greater coverts spots (n=68)				
Size of greater coverts spots	0.061	0.032	$F_{1,19}$=3.444	0.079
Number of median and lesser coverts (n=68)				
Size of median and lesser	0.676	0.537	$F_{1,19}$=1.579	0.224

Table SM 2.6: Results of the LMM analysing the relationship between the number and size of the spots in each patch in female common kestrels (*Falco tinnunculus*). Significant variables are in bold.

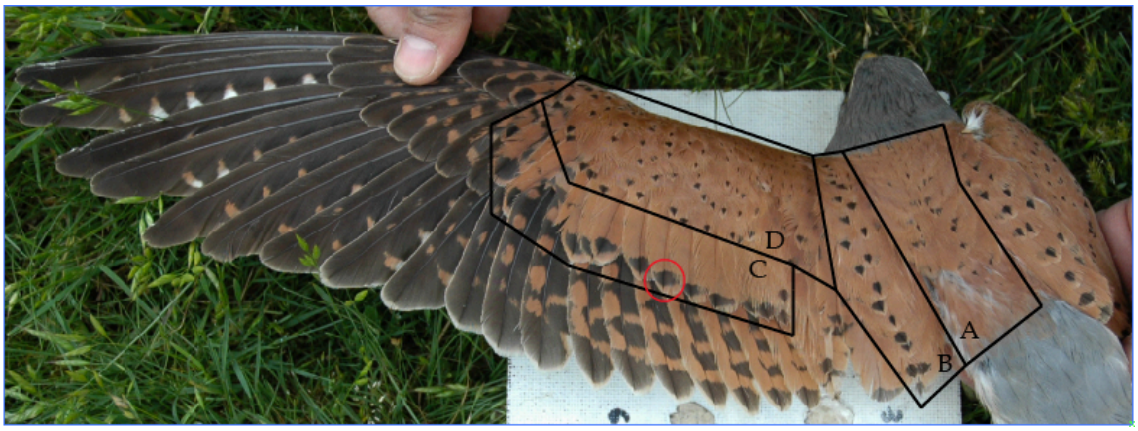

Figure SM 2.1: Common kestrel dorsum divided in four areas: (a) back, (b) auxiliary feathers (c) greater coverts and (d) median and lesser coverts. The red circle represents the measured spot of that area.

SM-2.2: Measured trait repeatability

Trait	Males		Females	
	R	P	R	P
Back spot size	0.742	**<0.0001**	0.711	**<0.0001**
Auxiliary feathers spot size	0.606	**<0.001**	0.210	0.100
Median and lesser spot size	0.337	**0.040**	0.390	**0.010**

Table SM 2.7: Results of the repeatability analysis done for the size of the measured traits in both males and females.

We also measured the repeatability (Lessells & Boag 1987; Nakagawa & Schielzeth 2010) of the measures that we took for both males and females. Our results indicate that only the measures of the size of back spots are repeatable enough. Considering what we stated below the number and size of both males and female common kestrel is the only melanin-based trait that we can measure efficiently. This is the main reason that we follow to use these traits in our analyses.

SM-2.3: Number of observations for males and females in each age class.

Age	2	3	4	5	6	7	8	9	Total
Males									
Reproductive traits	53	24	13	13	4	1	1	-	109
Ornamental traits	51	23	12	13	4	1	1	-	105
Females									
Reproductive traits	31	18	10	5	4	2	2	2	75
Ornamental traits	31	18	10	5	4	2	2	2	75

Table SM 2.8: Number of observations on each age class in males and females.

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Chapter III

Age but not food abundance modulates the expression of a signal of status in adults of female kestrel

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[1] Manuscript in preparation as: López-Idiáquez, D., Vergara, P., Fargallo, J.A. & Martínez-Padilla, J. (2016). Age but not food abundance modulates the expression of a signal of status in adults of female kestrel.

ABSTRACT

Females show conspicuous colourations that can work as status signals in a female-female competition context. These traits signal female competitive abilities, and their expression leads to the discrimination of dominant from subordinate individuals without eliciting agonistic encounters. Different studies have shown how signal expression can be modulated by different factors like age or environment. However, studies exploring how female badges of status change with age are less common. In this study, we take advantage of an individually-based dataset spanning 14 breeding seasons, to explore how female rump colouration of common kestrels (*Falco tinnunculus*) changes within individuals with age. Since the expression of melanin-pigmented traits can be modulated by prevailing environmental conditions, we also investigate the environmental influence on rump colouration, using vole abundance as a proxy of variation of environmental conditions. Our results show that the proportion of greyness of the rump increases within-individually as females age. Specifically, this increase mainly occurs between ages two and three, remaining stable from that age. We found that vole abundance, neither influenced the proportion of grey in the rump nor its association with age. Finally, our results show that rump colouration is negatively correlated to the number of offspring produced the previous years. We suggest that the enhanced expression of rump colouration with age may mean an increase in dominance of older females. The negative association between reproductive outcome the previous year and current trait expression suggests that rump colouration is costly to produce. According to the lack of environmental influence on the expression of this melanin-pigmented trait, we consider a higher cost of maintaining than producing this trait, perhaps paid in terms of social interactions due to its role as a badge of status.

INTRODUCTION

The presence of conspicuous colourations in females is widespread in nature, although the evolutionary forces driving their expression remain unclear (Tobias, Montgomerie & Lyon 2012). The expression of colourful traits results from both social or sexual competition (Amundsen 2000; Heinsohn 2005; LeBas 2006; Tobias *et al.* 2012). Within a social competition context, phenotypic traits can act as badges of status, allowing contestants to evaluate the competitive abilities of their opponents without eliciting agonistic encounters (Rohwer 1975). Alternatively, although not-mutually exclusive, the expression of conspicuous traits can be the result of sexual selection where the expression can have a signalling function and a cost associated with its expression (Clutton-Brock 2007; 2009) or non-functional by-product of shared genetic architecture with males (Lande 1987; Roulin *et al.* 2001; Potti & Canal 2011). Regardless of the evolutionary causes that determine the expression of sexual traits in females, its expression may impose a cost on the bearers, altering the resolution of trade-offs with other energetically demanding functions (Gustafsson, Qvarnström & Sheldon 1995; Møller 1989; Griffith 2000). These costs can be, for example, paid in terms of reproductive output (Fitzpatrick, Berglund & Rosenqvist 1995) or parasite load (Martinez-Padilla *et al.* 2011). However, it is required an understanding of the factors that explain the expression of sexual traits in females to accurately comprehend their potential function in animal communication. Here, we explored the effect of food availability and within-age variation as factors modulating the expression of melanin-based badges of status in females.

Age is a key a key factor modulating trait expression, as individuals physiology and ability to obtain resources change between different life stages (McComb *et al.* 2001; Nussey *et al.* 2011; McComb *et al.* 2011), altering the investment of limited resources to different energetically demanding functions throughout the life of individuals (Kokko 1997). According to life-history theory, is expected that as individuals age and their residual reproductive value decreases, they will increase their investment in signalling (Kokko 1997). Alternatively, individuals can also exhibit senescence and present reduced traits during the last life-stages, due to a deterioration in their physiological state over time, caused for example by a lower selection force in late-life (Hamilton 1966; Partridge & Barton 1996). However, the effects of senescence are not equally present nature, being more frequent in species that live longer (Ricklefs 1998; Turbill & Ruf 2010), which can make senescence difficult to detect in short-lived species.

Melanin is the most common pigment in animal integuments (McGraw 2006) and has an important function in animal communication (Jawor & Breitwisch 2003). For instance, enhanced expressions of melanin-based traits lead to the discrimination of dominant form subordinate individuals, evidencing the role of these traits as badges of status (Rohwer 1975; Møller 1987; Senar & Quesada 2006; Tibbetts 2006; Johnson & Fuller 2014). These traits can change with age, and adults usually express enhanced versions of the badges when compared to juveniles (Rohwer, Ewald & Rohwer 1981; Veiga 1993; Vergara *et al.* 2009; Potti, Canal & Serrano 2013). However, many of these studies have

focused on the differences between juveniles and adults (Rohwer *et al.* 1981; Veiga 1993; Vergara *et al.* 2009), despite there is also variation in the expression of these traits within the adult age class (Tella *et al.* 1997; Dreiss & Roulin 2010; Evans, Gustafsson & Sheldon 2011; Potti *et al.* 2013; Potti, Canal & Camacho 2014). Different studies have shown that there is an increase in dominance as individuals age (Piper & Wiley 1989; Stahl *et al.* 2001; Verhulst *et al.* 2014), and along the increase of the expression of melanin-pigmented trait in females, it suggests that they can play a role as badges of status. Only three studies have explored the within-individual variation of the expression of melanin-pigmented traits (Evans *et al.* 2011; Potti *et al.* 2013; 2014) using the white forehead patch and the mantle colouration of female pied (*Ficedula hypoleuca*) and collared flycatchers (*Ficedula albicollis*). The forehead patches of the flycatchers are depigmented rather than melanin-based traits and the mantle colouration is not a social signal, leaving the age-dependence of the melanin-based signals of status underexplored.

Despite that melanin-based colourations have a strong genetic basis (Roulin & Dijkstra 2003; Hubbard *et al.* 2010; Kim *et al.* 2013), the role of environmental conditions in the expression of melanin-based traits is widely accepted (Veiga & Puerta 1996; Fitze & Richner 2002; Fargallo *et al.* 2007a; López-Idiáquez *et al.* 2016b). For instance, previous studies have described that factors like parasite load (Fitze & Richner 2002) or food availability modulate their expression (Veiga & Puerta 1996; Fargallo *et al.* 2007a; López-Idiáquez *et al.* 2016b). This is of particular importance when the investment priorities of the individuals change, for example throughout their lives. Under poor environmental conditions old individuals should invest proportionally more in ornamentation than young individuals, as they have reduced future fitness prospects (López-Idiáquez *et al.* 2016b). Otherwise, under favourable environmental conditions the condition dependence of the ornamentation weakens, allowing both young and old individuals to display enhanced traits without any negative fitness consequence (David *et al.* 2000; Fargallo *et al.* 2007b; Cothran & Jeyasingh 2010; Vergara *et al.* 2012).

Here, we have explored the within-individual effects of age, the environmental conditions and their interaction on the expression on female rump colouration using common kestrels (*Falco tinnunculus*) as study species. Rump coloration works as a badge of status within the signalling system of kestrels (López-Idiáquez *et al.* 2016a) and is also an index of individual quality (Vergara *et al.* 2009). Previous studies have shown that the expression of this trait is associated to age, with an increased expression in adult (≥2-year old) than in juvenile individuals (one-year old; Vergara *et al.* 2009). However, as they did not disentangle the within- from the between-individual effects of age, the possibility that demographic heterogeneity hides a within-individual trajectory exists (Bouwhuis *et al.* 2009; Vaupel & Yashin 1985; van de Pol & Wrigth 2009). Therefore, we explored how the expression of rump colouration changes from a within-individual perspective (van de Pol & Verhulst 2006; van de Pol & Wrigth 2009). This approach allows us to identify, whether the age-dependence is caused because individuals increase their signals as they age, or by a differential mortality or arrival of individuals to the population with a certain trait expression.

Although previous studies have suggested that environmental variability can also modulate the expression of rump colouration, the specific environmental factors remain unknown (Vergara *et al.* 2009; Kim *et al.* 2013). In this study, we use common vole (*Microtus arvalis*) density as an environmental proxy of environmental variation. Voles are a good proxy of the environmental conditions, as kestrel population size varies associated with vole density (Fargallo *et al.* 2009), they are a major component of kestrels diet (Navarro-López, Vergara & Fargallo 2014), and the expression of kestrels melanin-based traits is sensitive to the variations of this prey species (López-Idiáquez *et al.* 2016b). The specific objectives are to explore the within-individual variation of rump colouration with age and with vole density and their interactive effect. We predict: (i) a within-individual positive association between age and the percentage of grey colouration on the rump, (ii) a positive relationship of rump colouration and the abundance of voles, and (iii) a stronger association between rump colouration and age under harsh environmental conditions.

METHODS

Study species

The common kestrel is a medium sized diurnal raptor that exhibits a marked sexual dimorphism in body size (females are 20% heavier) and plumage colouration (Village 1990; Palokangas *et al.* 1994). Females are brown on the head, back and upper sides of the wings, with black bars and display a variable amount of grey colouration on the rump (Vergara *et al.* 2009). In this species, moult usually takes place after breeding. Kestrels are characterized for being short-lived animals, that start breeding early and produce a relatively large number of young per breeding attempt (Village 1990). Specifically, in our population the maximum recorded life span of a female is 9 years old, but female with ages between 1 and 3 years old represent 80% of our population.

Study area

The study was conducted in Campo Azálvaro (40º40'N, 4º20'W) a treeless and flat area located in central Spain (1300 meters a.s.l.) mainly devoted to cattle-raising. In this area between 24 and 45 kestrel pairs breed each year in 62 nest-boxes progressively installed from 1994 to 2005 (Fargallo *et al.* 2001; 2009).

Data collection

The study took place between 2002 and 2015. During the breeding season, nests were monitored to detect laying date (the day that first egg was laid), clutch size (mean=5, range=3-6, n=101) and number of fledglings (mean=4, range=0-6, n=101). Females were captured using a net during incubation, and body mass (to the nearest g), wing and tarsus

length (to the nearest mm) were recorded. We determined rump colouration as the percentage of grey covering it (0% corresponded to completely brown rumps and 100% to completely grey rumps) as previously done in Vergara et al. (2009). We also determined whether females were yearlings (1 year old) or adults (≥2 years old) using ring codes or plumage features (Vergara & Fargallo 2007).

Environmental variables

As a proxy of food availability, we used the abundance of common voles (*Microtus arvalis*), a key component in kestrel diet (Navarro-López *et al.* 2014). Vole abundance was assessed by two trapping seasons each year carried out in autumn and spring. We set out 100 Sherman traps in four different plots (25 traps each) that were monitored six times for three days, three in the early morning and three at sunset. Trappings took place during new moon periods to avoid the effects of moonlight on small mammal activity (for further information see Fargallo *et al*. (2009).

Statistics

Within-individual change of rump colouration

We followed the method described in van de Pol *et al.* (2009) to explore the within- and the between-individual age effects in the expression of rump colouration. The within-individual term is calculated by subtracting the mean age of an individual from each individuals age value (Within-Individual Age-WIA=$xij - xj'$, where xij is the age-value of individual j at year i, and xj' is the mean age of individual j in the dataset; van de Pol & Wrigth 2009). We also used the age at first measurement (AFM) to control for the selective appearance of individuals in our population (van de Pol & Verhulst 2006). Finally, and to control for the potential effects of an age-mediated pattern of mortality, we calculated the Age at Last Measurement (ALM; van de Pol & Verhulst 2006). We fitted WIA, AFM, ALM and interactions between them as fixed factors, in addition in order to detect for non-linear patterns we also included their quadratic terms. As dependent variable, we included the year-centred value of the rump coloration in a Linear Mixed Model (LMM) with a normal error distribution. Female identity was included as random factor. We included breeding attempts from 2002 to 2014 in the analyses and used the data of 2015 to confirm that the non-breeding females of 2014 disappeared instead of having skipped one breeding season, improving the accuracy of the ALM.

The changes in the proportion of the greyness of the rump and WIA take place mostly during the first years of life (see results), particularly from the years 2 to 3, and seems to remain constant after that (see Supplementary Material-3.1). Thus, we explored the association between WIA and greyness of the rump considering two age periods, first from age 2 to 3, and second from 4 to 6. The models performed included the same variables than when considering all ages as described above.

Association between rump colouration and breeding variables

We performed 3 blocks of models to explore the association between rump colouration and laying date (LD), and two proxies of reproductive output, clutch size (CS) and number of fledglings (NF). In birds LD is a predictor of reproductive success as earlier breeders usually produce more recruits (Steenhof & Heath 2013) and have higher return rates (Steenhof & Heath 2009). In order to achieve normality in the analyses we used the year-centred value of the clutch size (CSc).

Firstly, we studied the association between rump colouration and reproductive output. We performed three separate models that included rump colouration as explanatory variable. Two of them were LMMs with normal error distribution, and LD and CSc as dependent variables, the third one was a Generalized Linear Mixed Model (GLMM) with Poisson distribution of errors and NF as dependent variable. All models included female age as a covariate and identity as random factor, year was also included as a random factor in the models where LD and NF were the dependent variables.

Secondly, to test the potential relationship between reproductive effort in $year_{(t)}$ and signalling during $year_{(t+1)}$, we explored the association between rump colouration and previous years' reproductive variables. We fitted three LMMs with normal distribution of errors that included rump colouration as dependent variable and previous years $LD_{(t-1)}$, $CS_{(t-1)}$ and $NF_{(t-1)}$ as explanatory. In all models $age_{(t)}$ was included as a covariate and female identity and year as random factors.

Finally, to explore the association between current investment in signalling and future reproductive output we tested whether there was an association between the proportion of grey rump coloration and following years' reproductive variables. With that aim we included LD, CSc and NF as dependent variables in 3 different models in which rump coloration in the previous year was included as explanatory variable. The models including LD and CS were LMMs with normal error distributions and the model including NF was a GLMM with Poisson distribution of errors. Female age was included as a continuous covariate in all models. Female identity was included as a random factor in all models and year in the models that included LD and NF as dependent variables.

Environmental influence on rump coloration

We used the number of voles in the previous spring (NVPS) as a proxy of the environmental conditions as previously described (López-Idiáquez *et al.* 2016b). NVPS was included as an explanatory in a LMM with normal distribution of errors in which the year centred value of rump coloration was the dependent variable. WIA was included as a covariate to control for the age-related change of the rump coloration and individual identity was included as a random factor. However, this analysis does not disentangle the within- from the between-individual environmental effect. To tackle this issue, we grouped NVPS in 5 categories according to their respective quartiles. Then we used these

categories to calculate the within-individual value of the NVPS (Within-individual "Vole"-WIV) in the same way that we calculated WIA. Then we fitted a new LMM including WIV as explanatory and the year centred value of the rump colouration as dependent variable. In these model, female identity was included as random factor.

In order to explore the environmental effect on the relationship between age and reproduction, we categorised the NVPS into high and low depending if they were above or below the respective overall mean (NVPSc). Due to sample size limitation, we also categorized WIA (WIAc) in levels (-1, 0 and 1) representing early ages (-1: WIA<0), midlife ages (0: WIA=0) and late life (1: WIA>0). Rump coloruation was z-transformed (RCz; mean=0 and sd=1) for the categories of NVSPc and WIAc. We also centred the reproductive values to the mean (laying date: LDc; clutch size: CSc and Number of fledglings: NFc) for each category of NVSPc and WIAc, that were included as dependent variables in our models. As explanatory variables, we included RCz, WIAc and NVSPc and the interaction between them. Individual identity and year were included as random factors.

In all models only adult females (≥2 years) of known age were considered. During the statistical analyses, we followed a backwards-stepwise selection procedure, in which all terms were initially included and then non-significant terms (p>0.05) were sequentially removed. All analyses were carried out in R statistical software using the packages *lme4* and *lmerTest* (Kuznetsova, Brockhoff & Christensen 2013; Bates *et al.* 2015).

RESULTS

Within-individual change of change of rump colouration

Our results show a significant positive correlation between female rump greyness and the within-individual age component (Table 3.1, Figure 3.1). However, no significant associations were found neither for AFM or ALM, nor for the interactions between the terms (Table 1). On the other hand, we have also found a positive correlation between rump coloration and WIA during early years of life (age 2 to 3; Table 3.2), but not during late life (age 4 to 6; Table 3.3).

Parameter	Estimate	SE	*F*	*P*	*E. Seq.*
Rump colouration					
WIA	**3.797**	**1.190**	**$F_{1,50.79}$=10.184**	**0.002**	
AFM	-5.674	4.792	$F_{1,53.91}$=1.401	0.241	7
ALM	-0.211	2.200	$F_{1,50.72}$=0.009	0.932	6
WIA^2	-1.918	1.108	$F_{1,54.58}$=3.194	0.079	8
AFM^2	-1.996	10.511	$F_{1,54.44}$=0.036	0.850	1
ALM^2	3.047	1.882	$F_{1,41.65}$=2.620	0.113	4
WIA*AFM	1.060	3.580	$F_{1,47.21}$=0.087	0.768	2
WIA*ALM	-1.272	1.243	$F_{1,48.61}$=1.047	0.311	3
AFM*ALM	7.151	4.891	$F_{1,44.8}$=2.137	0.150	5

Table 3.1: Results of the individual levels models of the expression of rump colouration in adult females (≥2 years) of known age. Rump colouration was centred to the year (see Methods). Within-individual age (WIA) represents individual change during the life of a given individual. Age at first measurement (AFM) represents the first reproduction in our population. Age at last measurement (ALM) is a proxy of longevity. In the models, female identity was included as a random factor. Variables in the final model are in bold; values for excluded variables refer to the step before their exclusion (E.Seq.)

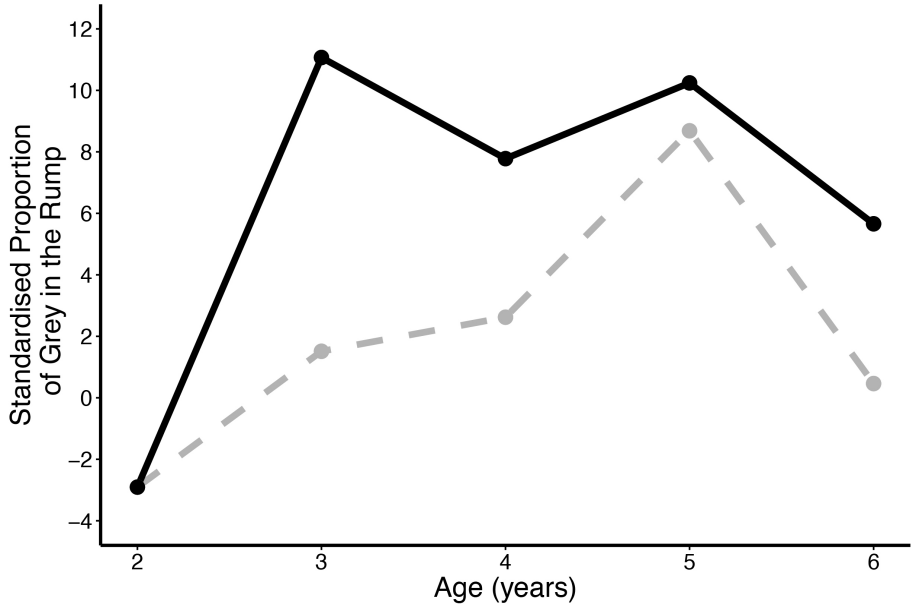

Figure 3.1: Patterns of standardized rump colouration (see material and methods for further details) in adult females. The black thick represents the within-individual change and the grey dashed line represents the population level pattern (see also SM 3.1). To perform this graphic we followed (Rebke *et al.* 2010).

Parameter	Estimate	*SE*	*F*	*P*	*E. Seq.*
Rump colouration					
WIA	**8,863**	**2.685**	**$F_{1,39.92}$=10.89**	**0.002**	
AFM	-4.829	5.064	$F_{1,53.39}$=0.717	0.400	5
ALM	1.153	2.499	$F_{1,63.22}$=0.212	0.646	4
WIA*AFM	7.224	6.109	$F_{1,25.81}$=1.398	0.247	2
WIA*ALM	0.690	2.859	$F_{1,35.20}$=0.058	0.810	1
AFM*ALM	7.348	5.239	$F_{1,47.93}$=1.402	0.167	3

Table 3.2: Results from the individual level of expression of rump colouration in adult females aged 2 and 3 years old. Within-individual age (WIA) represent the individual change in the expression of the trait during the life of a given individual. Age at last measurement (ALM) is a proxy of longevity. Age at first measurement represents the first reproduction in our population (AFM). In the models, rump coloration was mean-centred to the year and female identity was included as random factor.

Parameter	Estimate	*SE*	*F*	*P*	*E. Seq.*
Rump colouration					
WIA	1.746	3.371	$F_{1,7.77}$=0.268	0.619	5
AFM	7.793	7.843	$F_{1,12.70}$=0.987	0.339	
ALM	1.722	4.607	$F_{1,9.83}$=0.139	0.717	4
WIA*AFM	6.482	15.101	$F_{1,6.23}$=0.184	0.682	2
WIA*ALM	-11.117	7.000	$F_{1,7.84}$=2.568	0.152	3
AFM*ALM	4.723	17.317	$F_{1,10.58}$=0.074	0.790	1

Table 3.3: Results from the individual level of expression of rump colouration in adult females aged 4, 5, and 6 years old. Within-individual age (WIA) represent the individual change in the expression of the trait during the life of a given individual. Age at last measurement (ALM) is a proxy of longevity. Age at first measurement represents the first reproduction in our population (AFM). In the models, rump colouration was mean-centred to the year and female identity was included as random factor.

Association between rump colouration and reproductive output

In the first set of models, we did not find any significant correlation between rump colouration and reproductive output, neither for LD ($F_{1,56.14}$=0.057, P=0.811), nor for CS_c ($F_{1,81.68}$=1.037, P=0.288), or NF (-0.004±0.002, F=2.901, P=0.088). Female age was not significant in any of the three models (p>0.131).

In the second set of models we found a significant negative association between $NF_{(t-1)}$ and rump colouration (-1.932±0.811, $F_{1,45.50}$=5.672, ***p*=0.021;** Figure 3.2). No significant relationships were found for $LD_{(t-1)}$ ($F_{1,59.58}$=2.145, *p*=0.148) or $CS_{(t-1)}$ ($F_{1,62.88}$=0.048 *p*=0.827). In all models, age was retained as a significant factor (p<0.017).

Finally, we did not find any significant association between reproductive output and rump colouration of the previous year (Rump colouration-LD: $F_{1,65.60}$=0.760 *p*=0.386; Rump

colouration-CSs: 0.004±0.002, $F_{1,71.12}$=3.326, p=0.072; Rump colouration-NF: $F_{1,72.00}$=0.840, p=0.362). In this set of models, age was not significantly associated with reproductive variables (p>0.147).

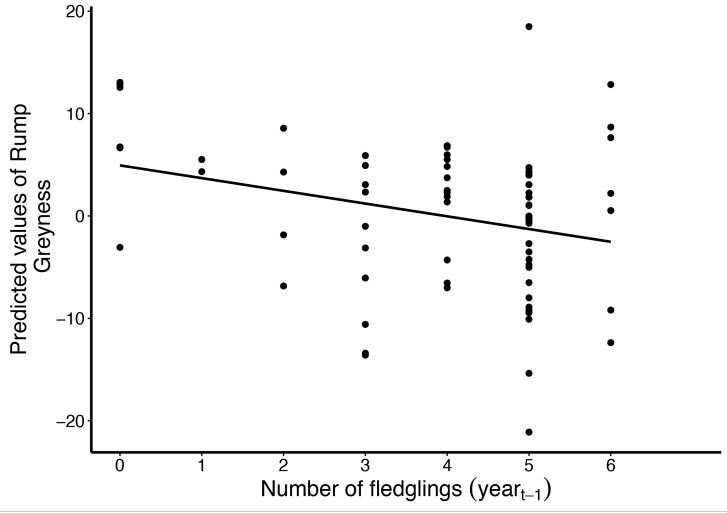

Figure 3.2: Relationship between the percentage of grey rump colouration and the number of fledglings breed the past year. In the y-axis, we represent the residuals of the models.

Environmental influence on rump colouration

In our first set of models, we did not find a significant correlation between rump colouration and NVPS ($F_{1,79.95}$=1.210, p=0.274). WIA was retained as a significant covariate (3.797±1.190, $F_{1,\ 50.79}$=10.184, ***p*=0.002**). In addition, rump colouration and WIV were not significantly correlated ($F_{1,49.17}$=1.078, p=0.304). Finally, none of the reproductive variables were explained by the triple interaction between WIAc*NVPSc*RCz (LDc $F_{2,80.230}$=0.427 p=0.653; CSc: $F_{2,83.595}$=0.032 p=0.968; NFc: $F_{2,89.0}$=2.537 p=0.084).

DISCUSSION

We used a database spanning 14 breeding seasons to investigate the influence of age and environment on the expression of adult female kestrel rump colouration. We show that a within-individual increase of the proportion of grey in the rump as females age. However, the increase takes place mainly during the ages 2 and 3 (Fig. 3.1). We also found that the amount of grey colouration in the rump is negatively associated with the number of fledglings the previous reproductive season. Still, we did not find any significant relationship between rump colouration and our environmental proxy (NVPS).

Our results evidence that the within-individual proportion grey colouration in the rump increases as females age. However, this association occurs during the ages two and three, and after that the expression of the trait remains stable. Kestrels are short-lived animals

that start breeding early in life (Village 1990), for example the 80% of the females in our population are 3 years-old or less (own data). This evidences that females face high selection pressures to breed during the first three years of their life, as the chances of survival of individuals older than 3 years are low. In kestrels, female rump coloration works as a signal of dominance in a female-female competition context (López-Idiáquez *et al.* 2016a), suggesting that the change in rump colouration between the ages two and three is concomitant with an increase in dominance. This is in agreement with other studies, which have found a positive association between age and dominance from both cross-sectional (Arcese & Smith 1985; Piper & Wiley 1989; Weiß, Kotrschal & Foerster 2011) and within-individual perspectives (Verhulst *et al.* 2014). Dominance improves through the participation in successive agonistic encounters that will increase individuals ability to fight (Arcese & Smith 1985). Thus, as individuals become more dominant in the first years of life, they will also have an increased resource availability and will be able to invest more in signalling (Arcese & Smith 1985; Stahl *et al.* 2001; Verhulst *et al.* 2014). Later in life, when individuals are older than 4 years old, the expression of the signal remains stable. It suggests that only females of prime quality are able to survive to those ages. Therefore, high-quality females are able to maintain an increased signal expression, explaining the low proportion of old individuals in the population. However due to our reduced sample size for old aged individuals we cannot rule out the possibility that a senescent pattern exists.

We have also found a significant negative association between the number of fledglings in $year_{(t)}$ and rump colouration in $year_{(t+1)}$. We suggest that this pattern is caused by the costs associated to an increased investment in reproduction, as previously suggested (Fargallo *et al.* 2007a). We did not find any statistical significant association between rump colouration and reproductive output in the same year, instead of the positive association between these two variables previously described in kestrels (Vergara *et al.* 2009). This divergence can be explained by the different approach used among studies. Our study only included adult individual (≥2 years), while Vergara *et al*. (2009) used both adult and juvenile females (one-year-old). The transition from youth to adulthood is when kestrels face the greater change in both ornamentation and reproductive output, explaining the differences in our results. Finally, we did not find any significant association between rump colouration in $year_{(t)}$ and reproductive output in $year_{(t+1)}$. This result suggests that female kestrels are able to face the potential cost of expressing greyer rumps from one breeding season to the following without any fitness consequences. Instead, the cost seems to be reflected on the greyness of the rump when females increase breeding effort the previous spring.

Previous studies have described that the extent of grey colouration in the rump has a high inter-annual variation (Vergara *et al.* 2009; Kim *et al.* 2013). However, these studies did not identify any particular environmental factor modulating the variation of rump colouration. According to our results, the inter-annual variation in the expression of rump colouration is not influenced by the NVPS, neither within a cross-sectional nor within-

individual perspectives. We suggest that the expression of this trait may have a production costs whose reliability is maintained by other means, like for example increased levels of aggression (sceptical receptor hypothesis; Rohwer 1977; Jarvi & Bakken 1984; Rohwer & Rohwer 1978). This is supported by a recent study showing that rump colouration works as a badge of status modulating aggressiveness in intra-sexual agonistic encounters (López-Idiáquez *et al.* 2016a). Finally, we did not find any significant result when testing the role of environmental conditions as drivers of the association between trait expression and fitness at different life-stages. This result evidences that the association of rump colouration and fitness is not modulated by our studied environmental proxy across the life of an individual.

In summary, our results show that the expression of rump colouration in females, a melanin-based trait, increases within-individually only during the first years of life. In addition, we also show that an enhanced reproductive investment reduces the expression of this trait the following breeding season. We also suggest that rump colouration may work as an honest signal of status and that the costs ensuring its reliability come from past reproductive investment or from the enhanced aggressiveness levels associated with its expression.

ACKNOWLEDGEMENTS

We thank J. Navarro-López and I. López-Rull for their help during fieldwork. The study was carried out on the Finat family property with the logistical support of J. San Teodoro. Licenses to study kestrels were granted by the Junta of Castilla y León. The long-term monitoring of the study population was financed by several projects of the Ministerio de Economía y Competitividad (CGL2004-04479/BOS, CGL2007-61395/ BOS, CGL2010-15726/BOS, CGL2013-42451-P) of the Spanish Government. This is a contribution of the El Ventorrillo Biological Station.

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SUPPLEMENTARY MATERIAL

SM-3.1

In spite that our results show that the proportion of grey colouration in the rump increases as females age, our graphical layout suggests that this change is mediated by the change between ages two and three. To test this, we fitted the points in the graph to both a linear ($y=a_1+a_2*x$) and to a half-life exponential ($y=a_1-(a_2/2^{\wedge(x/a_3)})$) distributions.

On the one hand, our results showed that neither of the two fitted distributions were statistically significant (Table SM 3.1, Figure SM 3.1), this may be caused due to our reduced sample size (n=5), however the exponential distribution presented a strong tendency to significance (p=0.08).

Distribution	*F*	*P*
Linear	$F_{1,2}=3.475$	0.165
Exponential	$F_{1,3}=10.814$	0.085

Table SM 3.1: Results of the goodness of fit of the values representing the within-individual change to a linear and a half-life exponential distribution.

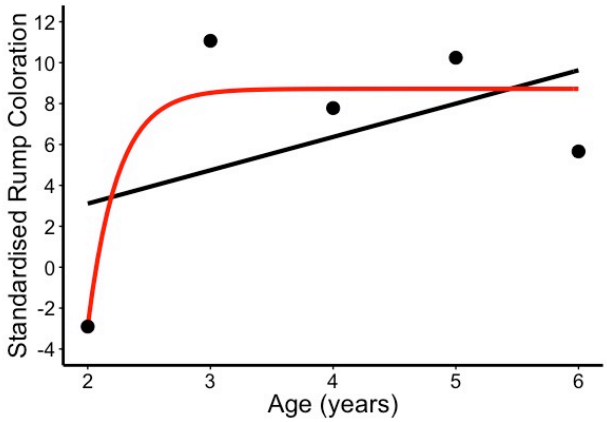

Figure SM 3.1: Age dependent pattern of rump colouration expression in adult females of known age. The points represent the values for the within-individual change and were obtained following (Rebke *et al.* 2010). The red line represents the fit to a half-life exponential distribution and the black line to a linear distribution.

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Chapter IV

Female plumage coloration signals status to conspecifics*

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[1] Published Manuscript: López-Idiáquez, D., Vergara, P., Fargallo, J.A. & Martínez-Padilla, J. (2016). Female plumage coloration signals status to conspecifics. *Animal Behaviour*, 121: 101-106.

*For consistency, we have used British English in this PhD dissertation, however this chapter is a literal transcription of the paper published in Ecology and Evolution, where American English was used.

ABSTRACT

Intrasexual competition in female birds is a widespread phenomenon but remains scarcely explored. Females compete for limited resources and the expression of signals that indicate their social status will be favoured by natural selection, generating a dominance hierarchy among individuals. However, which signals might be involved in female-female competition is unclear. In this study, we tested the role of rump coloration as a badge of status within a context of intrasexual competition using common kestrels, *Falco tinnunculus*, in two populations over 3 years. We used natural decoys with two different 'phenotypes', since the expression of this melanin-pigmented trait within the brown-grey range has been suggested to be a reliable proxy of individual quality in female common kestrels. By showing natural decoys with grey and brown rumps to breeding females, we simulated territorial invasions of high- and low-quality females, respectively. Our results show that rump coloration generates a differential response during territorial invasions. Specifically, we found that grey-rumped decoys (high quality) elicited lower levels of aggressiveness. In addition, female agonistic response was negatively associated with clutch size. Based on our results, we suggest that female rump coloration works as a badge of status in breeding female kestrels. This trait may signal female competitive ability and can generate a dominance hierarchy among individuals in our population. We also suggest that aggressiveness can be costly to females in terms of reproductive outcome. Overall, we provide evidence that melanin-pigmented traits in females can play a key role in intrasexual competition.

INTRODUCTION

Intrasexual competition in females is a widespread phenomenon in the animal kingdom, although its implications in behavioural and evolutionary ecology remain little explored (Stockley & Campbell 2013). Recent research suggests that females will compete for ecological resources, such as food or nesting sites, rather than for matings (LeBas 2006; Rosvall 2008; Tobias, Montgomerie & Lyon 2012), confirming the idea that female and male ornaments can be shaped by different selective forces (Rosvall 2011a). In a competitive context, the expression of signals that regulate access to limited ecological resources will be favoured by selection (Amundsen 2000). These signals, known as badges of status, allow contestants to evaluate the competitive abilities of other females without eliciting agonistic encounters, reducing the costs associated with aggressiveness (Rosvall 2011b). However, how females signal individual status and what phenotypic traits are involved in the wild are still poorly understood.

The reliability of signals of social status has been explained by different hypotheses (Senar 2003). First, signals of status are expected to be reliable proxies of individual status when they consistently have an associated behaviour, because receptors not only focus on the trait but also on the way individuals behave (sceptical receptor hypothesis; (Rohwer 1977; Järvi, Walsø & Bakken 1987; Rohwer & Rohwer 1978). Second, it has been proposed that only high-quality individuals are able to afford the cost of expressing an enhanced signal of status. This cost may arise from increased intrasexual aggression (social control hypothesis; (Rohwer 1977; Rohwer & Rohwer 1978), predation (differential predation hypothesis; Fugle *et al.* 1984; Fugle & Rothstein 1987) or immunosuppression (Folstad & Karter 1992). Finally, it has been suggested that dominant and subordinate individuals may follow alternative but evolutionarily stable strategies (mixed stable evolutionary strategy; Maynard Smith 1982; Rohwer 1982). This may be achieved, for example, if dominant and subordinate individuals allocate their resources differently, trading off resources to obtain high-quality territories and parental care (Studd & Robertson 1985; but see Yezerinac & Weatherhead 1997).

Within a social context (Senar 2006), melanin-based signals are particularly relevant in animal communication (Jawor & Breitwisch 2003). Several examples have described how an enhanced expression of melanin-based badges leads to the discrimination of dominant from subordinate individuals (Jarvi & Bakken 1984; Senar *et al.* 1993; Tibbetts 2006; Vergara, De Neve & Fargallo 2007; Rat *et al.* 2014; Johnson & Fuller 2014; Nakagawa *et al.* 2007) suggesting that these badges can act as social signals. Although these examples are biased towards males, there is growing evidence that females also show their social status since the expression of coloured traits mediates aggressiveness or dominance in females in various taxa (Midamegbe *et al.* 2011; Morales *et al.* 2014; Swaddle & Witter 1995; Murphy *et al.* 2009a; b; Crowhurst *et al.* 2012; Pham *et al.* 2014). There is also a bias towards studying carotenoid-based traits: for example, the size of the yellow patch in female rock sparrows, *Petronia petronia*, is a signal of their competitive ability (Griggio, Zanollo & Hoi 2010). However, the role of melanin-pigmented traits in

female intrasexual competition is little explored (Morales *et al.* 2014; Crowhurst *et al.* 2012). In fact, only two studies have examined the role of a lack of melanization (i.e. white spots), finding that white flank spots and forehead patch can signal status in diamond firetails, *Stagonopleura guttata*, and pied flycatchers, *Ficedula hypoleuca*, respectively (Morales *et al.* 2014; Crowhurst *et al.* 2012). Thus, how variation in melanization of female traits signals individual status is still poorly understood.

A first step to understanding how signals of status have been maintained in evolutionary time is to explore the costs associated with female-female competition. In females, aggressiveness has been directly linked to cuckolding avoidance (Gowaty 1981; Gowaty, Plissner & Williams 1989; Hobson & Sealy 1990) or to regulating access to nesting sites (Rosvall 2008). The related costs associated with this behaviour can be paid in terms of energy expenditure or physical damage (Cain & Ketterson 2013). As a consequence, this might constrain direct investment in reproduction or other energetically demanding functions. Thus, if expressing a signal of high status allows females to avoid the costs of agonistic encounters, it might be expected that those females will have more resources to allocate to other energy-demanding functions, such as reproduction (Knapton & Falls 1983; Kopachena & Falls 1993; Rosvall 2011b). Under this scenario, a positive selection of signals expressing social status in females is expected. However, our knowledge of the association between the expression of a social signal and levels of aggressiveness in females is extremely limited.

In this study, we simulated female territorial invasions by means of natural decoys to test female response within a context of intrasexual competition using common kestrels, *Falco tinnunculus*, as a study species. We presented decoys that differed solely in their expression of a melanin-pigmented trait: grey or brown rumps, representing high- and low-quality individuals, respectively (Vergara & Fargallo 2007; Vergara *et al.* 2009). Previous studies in this species suggest that female plumage is under ecological rather than sexual pressure (López-Rull *et al.* 2016). Our main objective was to test the role of this plumage as a badge of status in a female-female competition context. We predicted that low-quality (brown-rumped) invaders will receive more attacks and that more aggressive females will pay a cost in terms of reproductive output.

METHODS

Study Area

The study took place in two populations. Population 1 is in a mountainous grassland area located in Campo Azálvaro, Segovia (1300 m above sea level, 40º 40'N, 4º20'W) where 24-45 kestrel pairs breed each year in 62 nest-boxes installed progressively between 1994 and 2005 (Fargallo *et al.* 2001; 2009). Population 2 is located in a cropland area in Villalar de los Comuneros, Valladolid (700m above sea level, 41º32'N, 5º08'W) where 26-64 pairs breed each year in 100 nest-boxes installed in 2009 (Paz *et al.* 2013). Decoy

presentations (see below) were performed in populations 1 and 2 during the breeding seasons of 2012-2014 and 2013, respectively.

Study Species

The common kestrel is a medium-sized raptor with a marked sexual dimorphism in body size (females are 20% heavier) and plumage coloration (Village 1990; Palokangas *et al.* 1994). Females are brown on the head, back and upper sides of the wings, with black bars rather than spots (López-Idiáquez *et al.* 2016). The amount of grey coloration on the rump of females is variable, from completely brown (0% grey) to fully grey (100% grey; (Vergara *et al.* 2009). In our study, the average percentage of grey coloration in rumps of breeding females is 67.37% in population 1 (N=81) and 70.65% in population 2 (N=23).

Breeding Performance and Female Information

We monitored nests every 2 days to detect laying date (day that the first egg was laid; (Martínez-Padilla *et al.* 2004), and to record clutch size (population 1 mean=4.84, range 2-7, N=83; population 2 mean=4.65, range 3-7, N=26) and number of fledglings (population 1 mean=3.36, range 0-6, N=83; population 2 mean=3.03, range 0-6, N=26). Females were captured during incubation by netting. At the time of capture, body mass (to the nearest g), wing and tarsus length (to the nearest mm) of all females were recorded. The proportion of the greyness of the rump was also determined as previously described (Vergara *et al.* 2009): 0%, only brown coloration; 100%, only grey coloration. We also determined whether females were yearlings (1-year-old) or adults (≥2 years old) by using ring codes or plumage features (Vergara & Fargallo 2007).

Experimental Manipulation: Decoys and Rump Greyness

We exposed a natural decoy of an adult female to test whether there were differences in the aggressive response of breeding females to grey- or brown-rumped decoys during the pre-laying period. Decoys were stuffed dead kestrels obtained from recovery centres (see below) and were presented in two different treatments: grey or brown rumps. Rumps constitute unique structures obtained as a whole piece from dead kestrels, and could easily be fixed and removed from the decoys with a pin. A total of eight different decoys and 15 different rumps were used. To avoid a decoy-dependent effect on any of the variables used to assess aggressive response of females (see below), we randomly used different decoys and rumps.

Decoys were displayed on a 1 m tall stick at a distance of 10 m from the nestbox (following Vergara *et al.* 2007; Vergara & Fargallo 2007; Vergara, Martínez-Padilla & Fargallo 2012). The studied nests were chosen when a female was detected in its surroundings as a cue for later breeding (see Vergara *et al.* 2007). Decoys were always presented with the rump pointing towards the entrance of the nestbox. We removed the decoy if females did not return after 1h of observation. When females returned, we measured their aggressive behaviour for 10 min. Observations were carried out between

07:30 and 17:30 hours using binoculars (10x52) and a telescope (20-30x60) from a car located further than 200 m away in order to avoid interference with female behaviour. If the car was not a plausible option, we recorded female behaviour from the ground, at a similar distance to observations made from the car. When possible, we repeated observations of the same female (mean number of observations per female in a single year: 1.52 ± 0.09; range 1-3) but always over a minimum interval of 3 days between two consecutive observations. As soon as females laid their first egg, we stopped the observations at those nests.

Assessment of Aggressiveness

We assessed aggressive behaviour in three different ways. We first recorded whether or not there was an aggressive response once the female returned to the nest. Second, we quantified the aggressive intensity when females returned to the nest. We categorized this behaviour into five levels: '0' for no response, the individual was present but did not attack; '1' for attacks with no contact with the decoy, just alarm calls, flights, looping or hovering over the decoy; '2' for attacks with only one physical contact with the decoy; '3' for attacks with more than one contact with the decoy; '4' for situations in which females perched on the decoy and pecked it. Third, and finally, we recorded the number of attacks. These approaches have been successfully used in different studies with the same species in population 1 (Vergara *et al.* 2007; Vergara & Fargallo 2007; Vergara *et al.* 2012).

Ethical Note

The decoys and rumps used in this study were obtained from dead kestrels provided by Grupo de Rehabilitación de la Fauna Autóctona su Hábitat (GREFA) and CRAS Madrid-Viñuelas with permission of the Consejería de Medio Ambiente y Ordenación del Territorio of the Comunidad Autónoma de Madrid (10/253520.9/13). Kestrels were not killed to carry out this study and wild kestrels were not harmed by the agonistic encounters against the decoys or by the manipulation. Licences to study kestrels were provided by the regional government of Castilla y León (Expte: EP/CYL/105/ 2012; SENEP/SG/256/2013; EP/CyL/58/2014).

Statistical Analyses

All analyses were carried out in R statistical software using "*lme4*" and "*lmerTest*" packages (Bates, Maechler & Bolker 2013; Kuznetsova, Brockhoff & Christensen 2013). Female aggressiveness was coded for the three indices considered as follows for each observation: aggressive response (0: did not attack; 1: attacked); aggressive intensity (the five levels described above); number of attacks.

In a first set of models, we performed generalized linear mixed models (GLMM) to explore any potential influence of the decoys themselves on aggression levels. We included the three indices of aggressiveness used as dependent variables and the decoys

as explanatory variables. Distributions of errors were Poisson and binomial when the dependent variables were attack intensity and number of attacks and aggressive response, respectively, in all models. Second, we ran GLMM to study the influence of the treatment (grey or brown rump) on female aggressiveness. The three indices of aggressiveness were considered dependent variables in separate models. In these, treatment was included as a fixed factor and observation day (date the observation was made according to the Julian calendar), weight, rump coloration and age (young/adult) of the female as covariates. As previous studies have shown that male presence can affect females' willingness to initiate agonistic encounters (Jonart, Hill & Badyaev 2007) we also included male presence as a covariate in our models. Rump coloration, weight and observation day were standardized to a mean of 0. Third, we analysed the relationship between aggressiveness and reproductive output. We performed GLMMs considering the three proxies of aggression levels considered as dependent variables, and laying date, clutch size and number of fledglings as explanatory variables. Treatment and observation day were also included in the models to control for their effect on the dependent variables. The reproductive variables and observation day were also standardized to a mean of 0 before including them in the models. Coloration of female rumps and observation day were included as covariates.

All models included the study area and year as covariates and individual identity and nest as random factors. Model selection followed a backward-stepwise procedure in which all variables were initially included. Nonsignificant variables ($P > 0.05$) were removed sequentially. To explore whether the order of presentation of the two colours had any effect of habituation, we ran separate models for the three dependent variables of aggressive behaviour described above. In these models, order of presentation, treatment and their interaction were the explanatory variables, in addition to observation day as covariate. Nest and individual identity were considered as random factors. Distributions of errors for each model were those described above for each of the dependent variables.

RESULTS

Decoy Influence

We did not find any significant influence of a particular decoy on the results for any of the variables of aggressiveness (aggressive response: $P > 0.211$; aggressive intensity: $P > 0.132$; number of attacks: $P > 0.076$) or any effect of habituation of females to colours (all $P > 0.952$).

Decoy Rump Coloration

We did not find any statistically significant effect of either our treatment or any of the variables on aggressive response (Table 4.1). For aggressive intensity, we found a significant negative relationship with observation day (Table 4.1). Specifically,

aggressive intensity decreased as the breeding season progressed. Finally, our results suggest an effect of our treatment on number of attacks (Table 4.1, Fig. 4.1). Decoys with brown rumps were attacked significantly more than grey ones (Table 4.1). None of the aggressiveness variables were found to be significantly explained by body mass, age (1-year-old versus adult), rump coloration, study area or the presence of the male (Table 4.1).

Parameter	Estimate	SE	*F*	*P*	*ES*
(a) Aggressive response (*N*=70)					
Treatment	-1.304	0.853	F_1=3.681	0.126	7
% of Grey in attackers' rump	0.442	0.711	F_1=0.004	0.534	1
Weight	0.774	0.588	F_1=0.956	0.188	3
Study Area	1.252	1.469	F_1=0.516	0.394	4
Age	-1.054	1.347	F_1=0.433	0.433	5
Observation day	-1.241	0.727	F_1=8.002	0.087	
Year			F_2=1.002	0.380	2
Male presence	-1.694	1.223	F_1=2.682	0.166	6
(b) Aggressive intensity (*N*=70)					
Treatment	-0.466	0.313	F_1=5.691	0.099	**7**
% of Grey in attackers' rump	0.231	0.259	F_1=0.122	0.374	1
Weight	-0.569	0.585	F_1=1.236	0.330	2
Study Area	1.019	0.779	F_1=0.368	0.191	4
Age	-0.362	0.579	F_1=0.037	0.532	3
Observation day	**-0.640**	**0.252**	**F_1=8.516**	**0.011**	
Year			F_2=0.658	0.228	5
Male presence	-0.600	0.368	F_1=2.622	0.102	6
(c) Number of attacks (*N*=70)					
Treatment	**-0.620**	**0.284**	**F_1=10.83**	**0.029**	
% of Grey in attackers' rump	0.192	0.285	F_1=0.036	0.500	2
Weight	0.296	0.247	F_1=0.688	0.231	4
Study Area	0.524	0.569	F_1=0.928	0.357	6
Age	-0.597	0.698	F_1=0.102	0.390	1
Observation day	**-0.546**	**0.247**	**F_1=5.163**	**0.027**	
Year			F_2=1.409	0.288	5
Male presence	-0.308	0.402	F_1=0.569	0.443	3

Table 4.1: Results of the Generalised Linear Mixed Models (GLMM) of aggressiveness variables (aggressive response, aggressive intensity and number of attacks) in female common kestrels. ES denotes the exclusion sequence of nonsignificant terms of the model, age represent whether individuals where yearlings or adults. Variables included in the final models are in bold; values for excluded variables refer to the step before their exclusion. Greyness of the rump is a percentage of grey in the rump of females (see Material and Methods for further details).

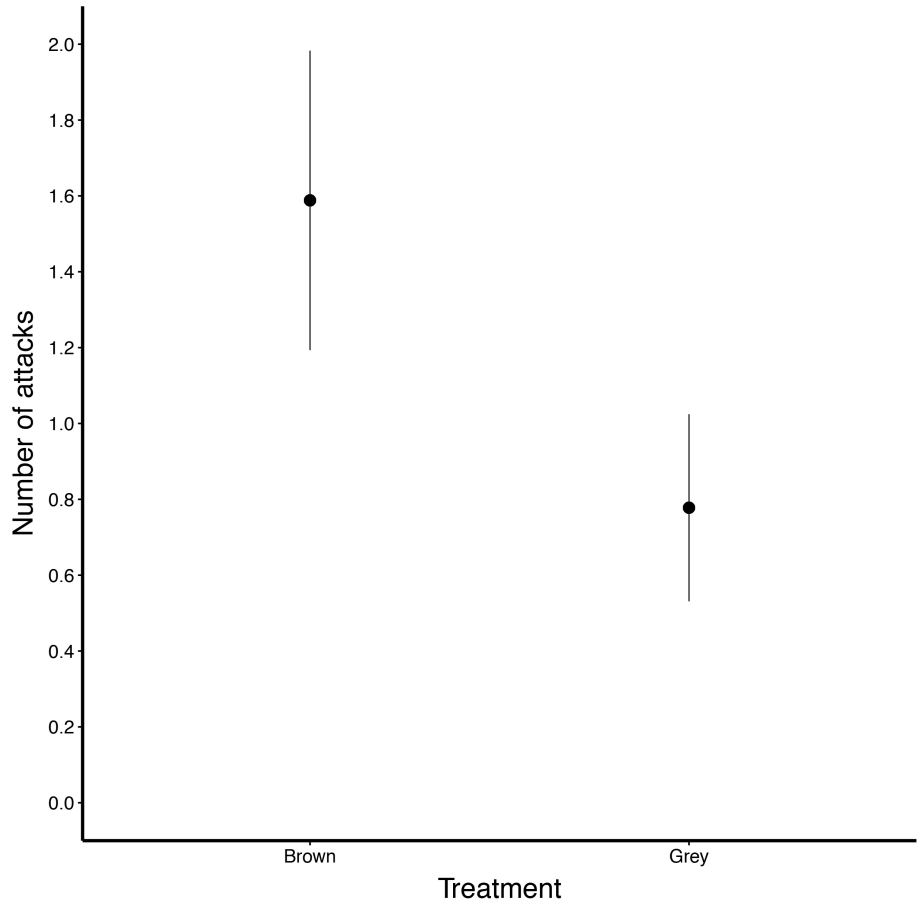

Figure 4.1: Number of attacks of adult female common kestrels on brown- and grey-rumped decoys. Means are given with SEs.

Reproductive Variables

Clutch size was negatively related to both number of attacks (GLMM: -0.628 ± 0.295, F=6.048, P=0.020; Fig. 4.2a) and aggressive intensity (GLMM: -0.573 ± 0.250, F=5.763, P=0.021; Fig. 4.2b). In relation to aggressive response, we found a marginally significant effect, where females that attacked had smaller clutch sizes (P=0.065). We did not find any significant relationship between any of the aggressive values and laying date (P > 0.135) or number of fledglings (P > 0.355).

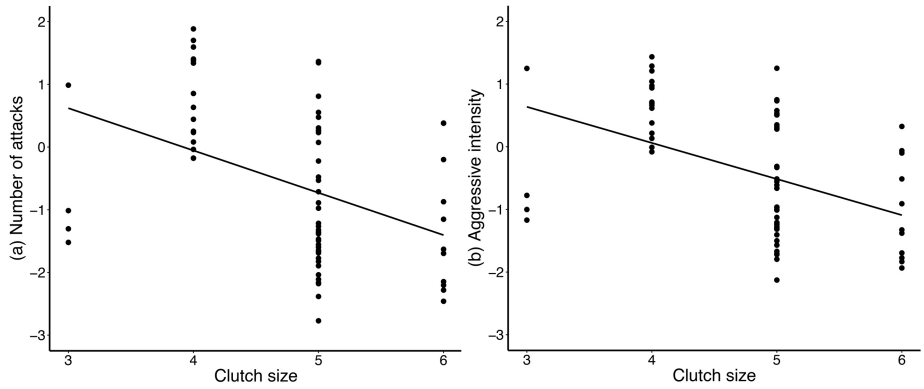

Figure 4.2: Relationships between clutch size and female aggressiveness. On the *y*-axis, we show the predicted values of the models performed in each case. (a) Number of attacks; (b) Aggressive intensity (see Methods for further details).

DISCUSSION

Our results suggest that rump coloration mediates the intensity of aggression of prelaying female common kestrels. Specifically, we found in a multiannual and spatially replicated experiment that females were more aggressive towards decoys with brown rumps. Further, we argue that aggressive behaviour can be a costly activity for females, since we found that female aggressiveness was negatively associated with clutch size.

The between-treatment difference found for the number of attacks indicates that female plumage coloration works within the signalling system of kestrels. This result agrees with previous studies in showing that female ornamentation can work as a status signal in a female competition context (Griggio *et al.* 2010; Morales *et al.* 2014; Crowhurst *et al.* 2012). These studies explored carotenoid (Griggio *et al.* 2010) and depigmented (Morales *et al.* 2014; Crowhurst *et al.* 2012) traits, making our study the first to investigate the role of melanin-based coloration in a female-female competition context. In addition, our results support the recent view that intrasexual competition for limited resources plays an essential role in the evolution of female phenotypes (Tobias *et al.* 2012). This is also supported by the fact that more feminized phenotypes, including plumage coloration, increase postfledging survival during winter (López-Rull *et al.* 2016). Our study indicates that females with greyer rumps can be perceived as more competitive individuals. Thus, plumage coloration can be considered a signal of social status that allows conspecifics to evaluate the probability of success in agonistic confrontations, as proposed by the status signalling theory (Rohwer 1975). Therefore, grey rump coloration, which is costly to produce (Fargallo *et al.* 2007) and reflects high individual quality (Fargallo *et al.* 2007; Vergara & Fargallo 2008; Vergara *et al.* 2009), may provide benefits within this hierarchical system of dominance in adult females. It is interesting, however, that, according to our results, rump coloration affects not female willingness to attack but the number of attacks. This result may suggest that females always respond to a territorial invasion and that it is the intensity of the response,

measured as number of attacks, that changes depending on the intruder's rump coloration. We do not have a clear explanation for this result, but it might be caused by a small sample size, although the two proxies of intensity of aggressiveness (considering only females that responded, i.e. aggressive intensity, or all females, i.e. number of attacks) have the same estimates, suggesting the same pattern. Possibly, a given threshold of stimulus is needed to trigger a more intensive response by females towards brown decoys.

Based on our results, we suggest that aggressive behaviour of females is an armament within an intrasexual competition context and probably under social selection. We base this statement on two main results. First, we found that aggressiveness of conspecifics was modulated by a badge of status, since greyer female decoys received fewer attacks from breeding females. Thus, showing an enhanced signal of status (higher proportion of greyness in the rump) is expected to prevent aggressive confrontations with other females. Second, our results suggest that aggressiveness can be considered a costly activity since it was negatively correlated with clutch size. This cost can be explained from different perspectives. According to the handicap principle (Zahavi 1975), only high-quality individuals are able to afford the cost of being more aggressive. Specifically, and according to our results, highly aggressive females are expected to be negatively selected because their aggressiveness would detract resources for reproduction (Fitzpatrick, Berglund & Rosenqvist 1995; Rosvall 2011b; Cain & Ketterson 2013) and only an intermediate expression of this trait would gain fitness benefits (Chenoweth, Doughty & Kokko 2006). Our results agree with this idea since more aggressive females produced smaller clutches, although our small sample size does not allow us to test the association between levels of aggression and clutch size at intermediate levels of aggression. Alternatively, the negative association between aggressiveness and clutch size can arise from an opposite perspective. One might expect that due to high reproductive costs (Stearns 1992), females laying smaller clutches can be more aggressive simply because they have more energy to invest. This is unlikely in kestrels because female body condition before and during laying is tightly associated with male quality since only males provide food to breeding females (Village 1990). Thus, the costs of being aggressive might be associated with mate quality. Under this idea, it is highly unlikely that females mated with low-quality males, laying smaller clutches, increase their aggressiveness, a costly activity for a low reproductive investment, at least in terms of clutch size. From a mechanistic point of view, testosterone levels may explain the association between clutch size and aggressive behaviour, as previous studies have shown that testosterone modulates aggressiveness (Jawor & Breitwisch 2003; Bókony *et al.* 2008) and is negatively associated with reproductive output (Rutkowska *et al.* 2005; Veiga & Polo 2008). In either case, from an evolutionary perspective, highly aggressive females are expected to be negatively selected. Surprisingly, previous studies in the same area (population 1) and with the same species found different results: clutch size was positively correlated with aggressiveness (Vergara & Fargallo 2007). However, the methodological approach is not comparable to our study. Vergara et al. (2007) used three decoys, including two males, so the aggressive response they reported cannot be linked specifically to a female-female encounter, as is the case in the present study.

We found that aggressiveness decreased during the breeding season. This result may be explained by the early arrival of better quality females to the breeding grounds. This can increase female competition for both obtaining and keeping a mate at the beginning of the breeding season (Village 1990; Wiklund & Village 1992). As the breeding season progresses, the number of single females and the potential risks of being cuckolded decrease, as does aggressiveness. This idea has been described for red-winged blackbirds, *Agelaius phoeniceus*: females develop a more aggressive response towards other females showing interest in mating with the territory-owning male (Yasukawa & Searcy 1982). Another nonexclusive explanation may be that the quality of territories and mates and, thus, the reproductive value of late-breeding females decrease as the breeding season progresses, and the cost of an aggressive behaviour might not compensate for maintaining low-quality territories or mates.

Overall, our results show that female rump coloration works as a badge of status in breeding female common kestrels. Greyer individuals of better quality (Fargallo *et al.* 2007; Vergara *et al.* 2009) were less intensely attacked than browner individuals. This indicates that kestrels are sensitive to variations in the expression of this colour trait (grey-brown rump coloration) and that rump coloration may signal status, at least in an intrasexual context. In addition, our study supports the idea that premating aggressiveness may impose costs observable in subsequent reproduction.

ACKNOWLEDGEMENTS

We thank Juan Navarro-López and Isabel López-Rull for their inestimable help during fieldwork and to the Finat family for allowing us to work on their property (Campo Azálvaro). Juan San Teodoro, Gregorio Villar and the City Council of Villalar de los Comuneros provided logistical support. This is a contribution of El Ventorrillo Biological Station. This study was financed by the Spanish Ministerio de Economía y Competitividad (Project: CGL2010-15726/BOS).

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Chapter V

Providing longer post-fledging periods increase offspring survival at the expense of future fecundity

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[1] Manuscript in preparation as: López-Idiáquez, D., Vergara, P., Fargallo, J.A. & Martínez-Padilla, J. Providing longer post-fledgling periods increase offspring survival at the expense of future fecundity.

ABSTRACT

The cost of reproduction is a key concept in life-history trade-offs. However, our understanding of the reproductive costs is biased towards measures of reproductive effort obtained before offspring independence. During the post-fledging dependence period (PFDP), parent birds feed and protect offspring, but the effort devoted to this reproductive activity has never been considered under the scheme of the costs of reproduction. Further, if costly PFDPs increase offspring fitness, it may emerge a potential parent-offspring conflict. However, the potential fitness benefits and costs for offspring and parents respectively of the duration of PFDP remain barely known. We estimated the duration of the PFDP during 5 years using wild common kestrels (*Falco tinnunculus*) and studied its association with recruitment probability and future parental reproductive performance. Our results show that longer PFDPs increased the probability of recruitment of fledglings, probably due to the benefits obtained by parental care. Also, we found that providing longer PFDPs was associated with reduced clutch sizes the subsequent breeding season in males. We suggest that increased reproductive investment on offspring during the PFDP may represent a cost of reproduction in breeding males, resulting in a life-history trade-off between current and future reproduction paid in terms of reduced clutch size. Given that longer PFDPs increases offspring survival, we suggest a parent-offspring conflict over the length of the PFDP. Overall, we speculate that a parent-offspring conflict might mediate the trade-off between current and future reproduction of breeding males in kestrels.

INTRODUCTION

Life-history theory aims to investigate the allocation of resources among different life-history traits (Stearns 1992). Since resources are limited, the classical view considers that life-history traits can be bound together promoting trade-offs among them, whose balance determines individual fitness. The presence of costs underlies the idea of trade-off, and multiple studies in wild conditions have identified different kinds of costs, that can modulate life-history trade-offs (Gustafsson & Sutherland 1988; Dijkstra *et al.* 1990; Gustafsson & Pärt 1990; Alonso-Alvarez *et al.* 2006; Metcalfe & Alonso-Alvarez 2010). Since the two components of fitness are survival and reproduction, it is unsurprising that the trade-off between current and future reproduction is one of the best studied in behavioural ecology in different taxa (Gustafsson & Sutherland 1988; Gustafsson & Pärt 1990; Candolin 1998; Hanssen *et al.* 2005; Rivalan *et al.* 2005; Hamel, Côté & Festa-Bianchet 2011). Particularly, the cost of reproduction implies that increasing current reproductive investment will reduce future reproduction or survival (Stearns 1992). Given the costs of parental care (Clutton-Brock 1991), adult breeders may be subject to reduced survival prospects or lower reproduction in future breeding attempts, (Stearns 1989; Wheelwright, Tice & Freeman-Gallant 2003). However, our understanding of the reproductive cost in birds is biased towards measurements of reproductive investment during the pre-fledgling stage (Gustafsson & Sutherland 1988; Dijkstra *et al.* 1990; Gustafsson & Pärt 1990; Owens & Bennett 1994). The time when fledgling start their independence is a crucial stage in the life-time of an individual that might greatly determine parental fitness (Martínez-Padilla *et al.* 2014). However, we know rather little about the potential cost that prolonged parental care at the beginning of offspring independence has on adult breeders, and therefore its potential to mediate life-history trade-offs.

The post-fledging dependence period (PFDP) is defined as the time between a chick's first flight and full independence (Mock & Parker 1997). During the PFDP, fledglings obtain parental care benefits in terms of increasing feeding (Vergara, Fargallo & Martínez-Padilla 2010) enhancing nutritional status (Emlen 1995; Ridley & Raihani 2007), competitive capacity (Vergara & Fargallo 2008) and improving foraging and flying skills (Davies 1976; Johnson 1986; Marchetti & Price 1989; Bustamante 1994; Wheelwright & Templeton 2003; Yoda, Kohno & Naito 2004), potentially improving survival prospects (Ridley & Raihani 2007; Grüebler & Naef-Daenzer 2010). While very few and recent examples confirm that higher parental effort results in a longer PFDP (Vergara *et al.* 2010; Arroyo, De Cornulier & Bretagnolle 2002), the potential reproductive cost of extended breeding investment paid later in life is scarcely explored (Wheelwright *et al.* 2003).

The length of the PFDP might be mechanistically explained by the resource competition hypothesis (Vergara *et al.* 2010) or by the ontogenetic hypothesis (Ferrer 1993; Muriel *et al.* 2015). The former, defends that fledglings remain dependent as much as possible to monopolise parental care (Vergara *et al.* 2010). The latter states that fledgling full

independence occurs as soon as they reach the optimum body condition (Ferrer 1993; Muriel *et al.* 2015). Theoretically however, parent-offspring conflict emerges because each offspring within a family demands an increased parental investment since it is more related to itself than to other siblings, while genetic similarity between parents and to all offspring do not differ (Trivers 1974; Kölliker *et al.* 2015). Such differential within-brood allocation that fledglings receive during the PFDP has been associated with phenotypic traits, such as melanin-pigmented plumage traits, sex or age (Vergara *et al.* 2010; Arroyo *et al.* 2002). These differences can arise due to differential parental investment according to the expected fitness return of each fledgling (Stamps 1990). For example, parents can invest more in the dispersing sex when there is a limited resource availability in order to avoid future competition (Ridley & Huyvaert 2007). Thus, in light of the parent-offspring conflict, it would be expected that an increased parental investment during the PFDP will increase survival prospects of offspring, but will be a reproductive cost paid for later in life for breeders, through reduced survival and/or future reproduction.

In this study, we explored the fitness consequences of the length of the PFDP of 315 fledglings of common kestrels (*Falco tinnunculus*) during 5 years. We also explore the reproductive costs of the length of the PFDP on parents in the subsequent breeding season. The duration of the PFDP is variable in kestrels (Dijkstra *et al.* 1990) and we base our predictions considering that PFDP is costly for breeding parents. We therefore predict that 1) that parents raising offspring with longer PFDPs will pay a reproductive cost, either on reproduction or survival; and that 2) longer PFDP will increase recruitment rates of fledglings.

METHODS

Study species

The common kestrel is a medium-sized diurnal raptor that exhibits a marked sexual dimorphism in body size and plumage colouration, being males a 20% lighter and displaying a more conspicuous plumage than females (Village 1990; Palokangas *et al.* 1994; Massemin, Korpimäki & Wiehn 2000). At the beginning of the breeding season, males perform courtship feedings increasing their hunting effort twice or more (Masman, Daan & Dijkstra 1988; Village 1990). The time when these courtship feedings begins has a major influence on both the time that females start laying the eggs, egg investment and in clutch size (Meijer 1988; Village 1990; Martínez-Padilla & Fargallo 2007; Martínez-Padilla *et al.* 2010). Food provisioning during the breeding season is done nearly exclusively by males (Village 1990; Vergara & Fargallo 2008; Boileau & Bretagnolle 2014; Tolonen & Korpimäki 1994) and mainly females incubate the eggs (Village 1990). Male food provisioning is maintained throughout nearly all the breeding season until the second half of the nestling stage when females start hunting, supporting males in feeding the brood (Village 1990).

Plumage of fledglings is similar to females and display a variable proportion of grey colouration on the rump, a trait that has been described as an index of quality and that is associated with the duration of their PFDP (Vergara & Fargallo 2008; Vergara *et al.* 2009). We consider the length of the PFDP as the number of days that fledglings remain in the surroundings of the nest (see below for further details). In our population, nestlings fledge on average at 31.3 days (Vergara & Fargallo 2008), and recruitment takes place during the first or second year of life (López-Rull *et al.* 2016). Individuals reproducing for the first time after those years are extremely rare (López-Rull *et al.* 2016).

General procedures

The study was conducted from 2005 to 2015 in Campo Azálvaro region, a flatland, grassland and treeless area located in central Spain mainly used for cattle grazing (40º40'N, 4º20'W). During the breeding season, nests were monitored every two days to detect laying date (day the first egg was laid) and to record clutch size (mean=4.97, range=2-7, n=357) and number of fledglings (mean=3.49, range=0-7, n=357). Adult breeders were captured when nestlings were 13 days old and at 25 days of age nestlings were weighted (to the nearest g) and blood sampled for molecular sexing (Fridolfsson & Ellegren 1999). In addition, we also measured the proportion of grey colouration on nestling rumps (0% corresponding to a completely brown rump and 100% to a fully grey rump) following Vergara *et al.* (2009). Nestlings were also marked with a combination of colour rings during 2005-2007 and 2012 and with PVC rings with a unique alphabetic code during the year 2013 to identify fledglings from long distances (Vergara *et al.* 2010). We carried out a food-supplementation experiment in 2006 (Vergara *et al.* 2010), and we did not include experimental individuals in this study.

PFDP duration, offspring recruitment and adult survival

The duration of the PFDP was estimated during the months of June, July and August for 5 years (from 2005 to 2007 and during 2012 and 2013) by identifying all fledglings present around the nest every two days. In our study area, fledgings are easily detectable from long distances. Breeding nest-boxes are placed alongside fences and fledging are usually located perching on them rather than on the ground. Fledgling identification was made from a car, using a telescope (20x60) between 7:30 and 19:30, at a distance enough to identify the coloured rings or to read the PVC rings without interfering with fledgling behaviour (see Vergara et al. 2008 for further information). We considered "day of independence" as the last day an individual was observed and the duration of the PFDP as the number of days since chicks were 32 days old (age at which nestling fledge (Vergara & Fargallo 2008) until the day of independence. Nest monitoring was prolonged for 7 days from the last detection of a given offspring to increase the accuracy of the PFDP duration estimation.

We used fledgling survival as a proxy of offspring recruitment. It was estimated by capturing all breeding adults, and identifying the individuals previously ringed as nestlings. This approach has been successfully used in several studies with this species (Kim *et al.* 2013; López-Rull *et al.* 2016). Our survival data included information from the recapture of the adults, a minimum of two years after the PFDP estimation (López-Rull *et al.* 2016). Our estimation of the duration of the PFDP can be considered misleading, mortality and independence during the PFDP. However, the higher mortality rates faces by a fledgling take place as soon as they become independent from their parents (Magrath 1991) not during the PFDP when they are fed and protected from predation by the parents. During this and for the length of the long-term monitoring of our population, we visit all nests and surroundings very often and predation potential predated individuals (feathers or other prey remains) are easy to spot due to the landscape composition of our area. During 15 years, we have detected an extremely low rate of predation during the post-fledging period (n= 9 out of 1737 nestlings ringed from 1998 to 2016). Thus, we consider that our way to estimate of the duration of the PFDP is highly reliable, as previously shown and used in our study area (Vergara & Fargallo 2008; Vergara *et al.* 2010). Adult survival was estimated by capturing the whole breeding population a year later and identifying their individual tags, as previously done in Dijkstra *et al*. (1990). It can be argued that our estimates of survival, using recruitment probability as proxy, can be considered biased because common kestrels show a marked sex-biased breeding dispersal, where males disperse further than females (Terraube, Vasko & Korpimäki 2015). We consider this possibility unlikely since dispersal distances in kestrels fall mostly within a range of approximately 20Km (Terraube *et al.* 2015) and we are confident that kestrels do not breed out this buffer area since we regularly search for breeding pairs well beyond this distance (Fargallo *et al.* 2001).

Statistical procedures

All models were conducted in R statistical software using "*lme4*" and "*car*" packages (Fox & Sanford 2011; Bates *et al.* 2015). We performed three sets of models. The first set of models was carried out to explore the association between the duration of PFDP experienced by breeding adults in a given year (*t*) and their reproductive output the following year (*t+1*). We used Linear Mixed Models (LMMs) with normal distribution of errors considering clutch size (CS_{t+1}), and number of fledglings (NF_{t+1}) as dependent variables in different models. Since breeding adults in a given year (*t*) may have raised offspring that differed in the length of the PFDP, we used the mean duration of the PFDP of the brood for each adult each year ($PFDPmean_t$) as an explanatory variable. In order to control for the potential effect of current reproductive effort, we included clutch size (CS_t) and number of fledglings (NF_t) in year (*t*) as an explanatory variable when CS_{t+1} or NF_{t+1} were the dependent variables, respectively. Laying date (LD_{t+1}) was also included in all models to control for the differences in reproductive performance across the breeding season. Previous studies in this species have shown that clutch size is positively associated with age in males (López-Idiáquez *et al.* 2016). To control for the potential effects of this variable we included minimum age ("Minage") as an explanatory variable.

We included "Minage" because we do not have the real age of all the individuals, and including the latter will cause a reduction in the number of observations included in the models. We calculated the Variance Inflation Factors (VIFs) among our explanatory variables and found no evidence of collinearity (all<1.17 – SM-5.1).

In the second set of models, we explored the association between the duration of the PFDP and adult survival to the following breeding season for males and females separately. We included adult survival as a dependent variable and $PFDPmean_t$ as an explanatory term, using GLMMs with a binomial distribution of errors. We also included clutch size at year *t* as a covariate to control for the potential effects of past reproductive investment on adult survival. Year was included as fixed and individual identity as random factor in the first and second set of models. In addition, males and females were analysed separately as they play different roles during breeding and thus they may experience different reproduction costs.

The third set of models aimed to explore the association between the duration of the PFDP and recruitment, using Generalised Linear Mixed Models (GLMM) with binomial distribution of errors. Recruitment (1 or 0) was the dependent variable and PFDP, chick sex, rump colouration and weight were included as explanatory terms. Interactions between PFDP and sex and PFDP and rump colouration were also included in the models. Female identity was included as random factor. We used female instead of nest because female quality may mediate the duration of the PFDP (Vergara *et al.* 2010) and because the same female produced offspring in different years.

RESULTS

We found that the mean duration of the PFDP for the kestrel fledglings was 15.25±0.40 days (n=315). In relation to breeding adults, we found that the length of the PFDP and clutch size the following year ($t+1$) were negatively associated (Table 5.1). No relationships were found either for NF_{t+1}, or for the reproductive variables explored in females (Table 5.1). We found no significant relationship between PFDP and adult survival (Table 5.2).

Regarding offspring, we found that the length of the PFDP was positively associated with the probability of offspring recruitment (0.067±0.034, F=1.942, *P*=0.049). Year was retained as a covariate as two of its levels were significantly associated with the probability of recruitment ($Year_{2006}$, p=0.040; $Year_{2013}$, *P*=0.044). None of the explanatory variables (chick sex: F=0.021, *P*=0.647; rump colouration: F=0.518, p=0.414; weight: F=0.761, *P*=0.380) or interactions (PFDP*chick sex: F=0.869, *P*=0.351; PFDP*rump colouration: F=0.395, *P*=0.535) explained the probability of recruitment.

	Parameter	Estimate	SE	*F*	*P*	*E. Seq.*
FEMALES (n=43)				**CS_{t+1}**		
	Mean PF	-0.014	0.028	$F_{1,34.38}$=0.254	0.617	3
	LD_{x+1}	-0.006	0.017	$F_{1,32.34}$=0.142	0.708	1
	$Year_{x+1}$			$F_{4,26.69}$=2.346	0.080	
	$Minage_{x+1}$	-0.045	0.080	$F_{1,36.84}$=0.312	0.579	4
	CS_x	0.071	0.173	$F_{1,31.44}$=0.169	0.683	2
				NF_{t+1}		
	Mean PF	0.009	0.063	$F_{1,22.29}$=0.024	0.877	1
	LD_{x+1}	-0.012	0.039	$F_{1,34.90}$=0.101	0.752	2
	$Minage_{x+1}$	0.182	0.179	$F_{1,39.88}$=1.032	0.315	4
	$Year_{x+1}$			$F_{1,18.55}$=0.575	0.684	3
	NF_x	-0.334	0.209	$F_{1,39.02}$=2.554	0.118	
MALES (n=29)				**CS_{t+1}**		
	Mean PF	-0.014	0.028	$F_{1,34.38}$=0.254	0.617	3
	LD_{x+1}	-0.006	0.017	$F_{1,32.34}$=0.142	0.708	1
	$Year_{x+1}$			$F_{4,26.69}$=2.346	0.080	
	$Minage_{x+1}$	-0.045	0.080	$F_{1,36.84}$=0.312	0.579	4
	CS_x	0.071	0.173	$F_{1,31.44}$=0.169	0.683	2
				NF_{t+1}		
	Mean PF	0.009	0.063	$F_{1,22.29}$=0.024	0.877	1
	LD_{x+1}	-0.012	0.039	$F_{1,34.90}$=0.101	0.752	2
	$Minage_{x+1}$	0.182	0.179	$F_{1,39.88}$=1.032	0.315	4
	$Year_{x+1}$			$F_{1,18.55}$=0.575	0.684	3
	NF_x	-0.334	0.209	$F_{1,39.02}$=2.554	0.118	

Table 5.1: Results of the Linear Mixed Models exploring the association between the mean duration of the post-fledging dependence period ($PFDPmean_t$) and the following years' ($Year_{t+1}$) clutch size (CS_{t+1}) and number of fledglings (NF_{t+1}). $Minage_{x+1}$ represents the minimum age for an individual on our population. Statistically significant variables are highlighted in bold.

	Females (n=86)				Males (n=77)			
				Survival				
Parameter	Estimate	SE	*F*	*P*	Estimate	SE	*F*	*P*
$PFDPmean_t$	0.039	0.061	F_1=0.436	0.516	0.092	0.074	F_1=3.328	0.217
Year			F_4=2.562	0.120			F_4=1.084	0.210
CS_t	-0.057	0.362	F_1=0.026	0.873	**1.310**	**0.528**	**F_1=8.220**	**0.013**

Table 5.2: Results of the Generalised Linear Mixed Models exploring the association between the mean duration of the post-fledging dependence period ($PFDPmean_t$) and survival to the following reproductive season ($t+1$). Year and clutch size (CS_t) are included as covariates in the models. Statistically significant variables are highlighted in bold.

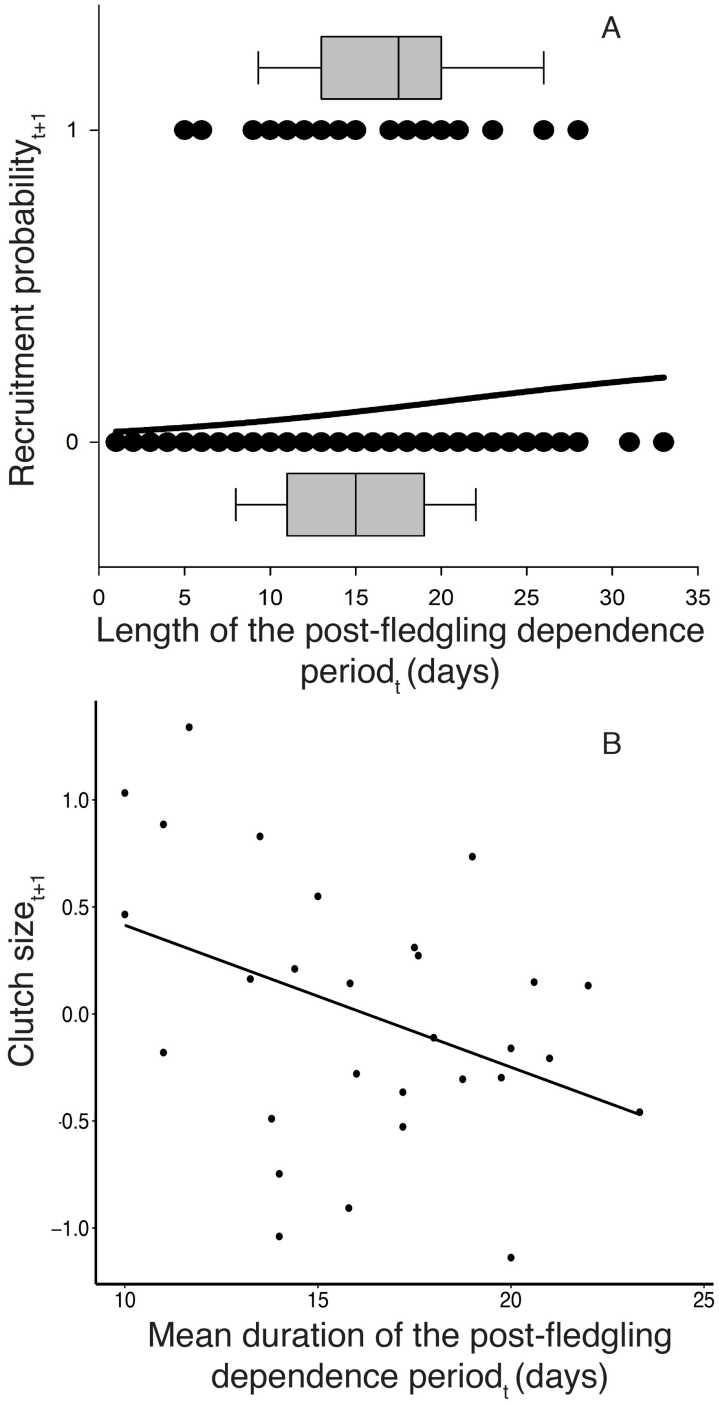

Figure 5.1: (A) Relationship between the probability of post-fledging recruitment rate and the duration of the post-fledging dependence period measured in days; and (B) the relationship between clutch size and mean duration of the post-fledging dependence period. Values of clutch size represents the predicted values obtained from the model (see methods for further details). In both figures, sub-indices denote current ($_t$) or following year ($_{t+1}$).

DISCUSSION

Our results show that the year after breeding offspring with longer PFDPs, males pair with females that lay reduced clutches, suggesting a reproductive cost. The cost of reproduction implies that increasing current reproductive investment will reduce future reproduction or survival (Stearns 1992). Our results support the idea that the cost of reproduction is paid in terms of future reproduction but not on survival to the following breeding season. This specific life-history trade-off between current and future reproduction has been previously demonstrated in birds (Gustafsson & Sutherland 1988; Gustafsson & Pärt 1990; Hanssen *et al.* 2005), explained by an increased energy investment during reproduction. However, we found a sex-dependent reproductive cost perhaps because of a higher investment of males than females in their offspring during the post-fledging period, likely in feeding and parental care (Naef-Daenzer, Widmer & Nuber 2001). This male-biased effect is not surprising in kestrels, since males are the main food providers throughout the breeding season, including the PFDP (Vergara & Fargallo 2008; Boileau & Bretagnolle 2014). In addition, it has been experimentally shown that only breeding males increase their hunting effort as a response to enlarged broods (Dijkstra *et al.* 1990). Thus, the reduced clutch size the following breeding season can be explained by a male cost paid when raising offspring for a longer PFDP the previous year. However, this cost vanishes when considering the number of offspring as a proxy of fitness instead of clutch size. Clutch size is a more reliable index of individual quality since larger clutch sizes will lead to more fledglings when environmental conditions are favourable. In our species, clutch size and the number of fledglings are highly correlated (Navarro-López & Fargallo 2015), but nestling mortality may be caused by stochastic factors (e.g., predation, weather conditions) between egg laying and chick fledging (Aparicio 1993), and clutch size is a better predictor of individual quality. In disagreement with previous studies, our results do not suggest that longer PFDP of parents reduces future survival, as previously reported in birds (Gustafsson & Sutherland 1988; Wheelwright *et al.* 2003) and also in kestrels (Dijkstra *et al.* 1990). Dijkstra *et al.* (1990) increased brood size in kestrels, imposing a stronger reproductive cost on adults that was translated into a lower survival. This difference may arise because the reproduction cost imposed on males by increasing brood size can be higher than for males raising offspring for longer PFDPs.

We found that the length of the PFDP increases recruitment probability of offspring. The association between the duration of the PFDP and recruitment may arise due to the benefits that offspring obtain during this period. For example, breeding parents in better body condition, are able to invest more time and energy in their offspring resulting in longer PFDPs (Vergara *et al.* 2010). This investment is expected to increase body condition of offspring (Ridley & Raihani 2007), a key component for recruitment and survival (Bouwhuis *et al.* 2015). Other factors, like the acquisition of hunting or flying skills, have also been shown to develop at the time of independence (Davies 1976; Johnson 1986; Marchetti & Price 1989; Bustamante 1994; Wheelwright & Templeton 2003; Yoda *et al.* 2004), potentially increasing survival prospects and acquisition of

breeding positions at a younger age (Ridley & Raihani 2007). Fledgling sex did not mediate the association between the length of the PFDP and recruitment rates. These results can be unexpected considering that females are superior competitors for food against their male sibs during the nestling period and better body condition at fledging increases offspring survival (Bouwhuis *et al.* 2015). In kestrels, during the nestling period, a lower competitive capacity of males results in a reduction in body condition respect to female fledglings (Dijkstra *et al.* 1990; Fargallo *et al.* 2003). However, there is a shift in the access for food provided by parents during the PFDP (Vergara & Fargallo 2008). Specifically, males outcompete females in their access for food due to their enhanced flying skills (Vergara & Fargallo 2008). These results may support the idea that there is a sex-dependent improvement in body condition in male fledglings during the PFDP, compensating a lower sibling competition abilities of males during the nestling period (Fargallo *et al.* 2003).

Taken together, our results indicate two opposing selective forces within a parent-offspring conflict context. In species that reproduce sexually, from an evolutionary perspective, parent-offspring conflict emerges because parental investment is unbalanced within members of the family: each offspring is expected to demand more investment from its parent since it is more related to itself than with other sib, while genetic similarity between parents and to all offspring do not differ (Trivers 1974). According to our results, offspring will be positively selected to maximize the duration of the PFDP because they increase their own probability of recruitment. Our results suggest that the amount of care (i.e., longer PFDP) provided to offspring may reduce a measure of individual fitness of the parent, suggesting a potential parent-offspring conflict over the length of the PFDP. Thus, we speculate that the duration of PFDP can be a source of parent-offspring conflict at least within a behavioural context that may mediate a life-history trade-off, particularly between current and future reproduction of breeding birds. Within an evolutionary context, our results are challenging since the number of offspring, understood as a proxy of Darwinian fitness, was not reduced. To accurately frame our results within an evolutionary perspective would require performing selection analyses. In order to explore the direction and type of selection, it would be needed to study the association between the length of the PFDP provided by the parents and different indexes of fitness over the years. Unfortunately, our data does not allow such analyses.

Previous studies have found that greyness of the rump is an index of individual quality in offspring of kestrels (Vergara & Fargallo 2008) since it is positively associated with body mass (Fargallo *et al.* 2007) and negatively with parasite species richness (Vergara & Fargallo 2007). Further, it has been suggested that greyer individuals have a higher probability to catch larger preys (Vergara & Fargallo 2008). Thus, in disagreement with our results, it is expected that offspring with greyer rumps had higher survival prospects. This lack of association can be explained because male kestrel fledglings with a more male-like phenotype (greyer rumps and smaller size) have worse survival prospects, than more feminized phenotypes (López-Rull *et al.* 2016).

Overall, our study suggests that reproductive investment on offspring during the PFDP may represent a cost, resulting in male-biased life-history trade-off between current and future reproduction. Given that longer PFDPs can be a reproductive cost that reduces male fitness but increases offspring survival, we suggest the existence of a parent-offspring conflict over the length of the PFDP. Overall, we speculate that a parent-offspring conflict might mediate the trade-off between current and future reproduction of breeding males.

ACKNOWLEDGEMENTS

We thank Juan Navarro-López, Isabel López-Rull and Juan San Teodoro for their help during fieldwork. The study was carried out on the Finat family property. Licences to study kestrels were provided by the Junta of Castilla and León. We thank Sarah Young for reviewing the English. This is a contribution of El Ventorrillo Biological Station. This work was supported by the Ministerio Economía y Competitividad (Projects: CGL2004-04479/BOS, CGL2007-61395/BOS, CGL2010-15726/BOS and CGL2015-70639-P).

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SUPPLEMENTARY MATERIAL

SM-5.1: Collinearity among the explanatory variables

Previous studies in this species have shown that the duration of the PFDP can be associated with reproductive effort within a given year (Vergara, Fargallo & Martínez-Padilla 2010). In this study, Linear Regression Models (LM) confirm this pattern considering PFDP as dependent variable and CS (estimate: 2.056±0.463, $F_{1,313}$=19.697, **p<0.001**) and NF (estimate: 1.218±0.348, $F_{1,313}$=12.218, **p<0.001**) as explanatory terms. Therefore, these variables may be collinear, claiming cautiousness at using them as explanatory variables. However, collinearity among explanatory variables cannot always lead to non-reliable results if their association do not inflate variances. To explore the association between associated variables, we calculated Variance Inflation Factors (VIFs) to test it (Zuur, Ieno & Elphick 2010). VIFs were calculated with "*vif"* function of *usdm* package (Babak 2015). All VIFs were smaller than 1.17 in female models and of 1.09 in males, suggesting a low influence of variables to inflate our results

	Models	
Variables	Clutch Size$_{t+1}$	N. of Fledglings$_{t+1}$
PFDPmean$_t$	1.082	1.001
Laying date$_{t+1}$	1.017	1.095
Clutch size$_t$	1.095	
N. of fledglings$_t$		1.094

Table SM 5.1: Variance Inflation Factors (VIF) for the explanatory variables used in the models exploring the association between the mean duration of the post-fledgling dependence period (PFDPmean) and followings year (*t+1*) reproductive output in males.

	PFDPmean$_t$	Laying date$_{t+1}$	Clutch size$_t$	N. of fledglings$_t$
PFDPmean$_t$	1	0.035	0.268	-0.006
Laying date$_{t+1}$		1	-0.112	-0.293
Clutch size$_t$			1	0.377
N. of fledglings$_t$				1

Table SM 5.2: Correlation coefficients between each pair of variables used as explanatory in the models exploring the association between the mean duration of the post-fledgling dependence period (PFDPmean) and followings year (*t+1*) reproductive output in males.

	Models	
Variables	Clutch Size$_{t+1}$	N. of Fledglings$_{t+1}$
PFDPmean$_t$	1.141	1.173
Laying date$_{t+1}$	1.091	1.098
Clutch size$_t$	1.090	
N. of fledglings$_t$		1.098

Table SM 5.3: Variance Inflation Factors (VIF) for the dependent variables used in the models exploring the association between the mean duration of the postfledgling dependence period (PFDPmean) and followings year (*t+1*) reproductive output in females.

	PFDPmean$_t$	Laying date$_{t+1}$	Clutch size$_t$	N. of fledglings$_t$
PFDPmean$_t$	1	0.235	0.235	0.275
Laying date$_{t+1}$		1	-0.107	-0.112
Clutch size$_t$			1	0.595
N. of fledglings$_t$				1

Table SM 5.4: Correlation coefficients between each pair of variables used as explanatory in the models exploring the association between the mean duration of the post-fledgling dependence period (PFDPmean) and followings year (*t+1*) reproductive output in females.

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Chapter VI

Bolder females show darker melanin-based traits in nestlings of common kestrel (*Falco tinnunculus*)

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[1] Manuscript in preparation: López-Idiáquez, D., Fargallo, J.A., López-Rull, I., & Martínez-Padilla, J. (2016). Bolder females show darker melanin-based traits in nestlings of common kestrel (*Falco tinnunculus*).

ABSTRACT

Animal personality is defined as the inter-individual differences in behaviour that are consistent across time and different contexts. The most common way to study individual differences in behaviour is the shy-bold axis, in which bold individuals are characterized to be more aggressive, and showing riskier behaviours than shy individuals. Variation in personality has been associated with differential expression of different coloured traits, particularly melanin-based traits. This relationship is supported because testosterone has been both associated with melanogenesis and aggressive behaviours. However, our knowledge of the association between melanin-pigmented traits and personality is biased to adults, despite offspring also shows variation in both the expression of melanin-coloured phenotypes traits and aggressiveness levels. Here we explored the association of personality and two melanin-based traits, plumage blackness and rump colouration, in 148 common kestrel nestlings during two years. We measured 5 behavioural traits that were combined in a principal component analysis (PCA). From the PCA analyses, a single axis represented a gradient of shy and bold behaviour that was associated with plumage blackness. Our results show that personality and plumage blackness are positively associated in females but not in males. No significant relationship was found for the association between rump colouration and personality. We discuss the possible sex-dependent role of melanin-pigmented traits as signals of individual personality both during the nestling and post-fledging dependent period. According to these results, we suggest that offspring's plumage blackness is a signal of personality that may be working within a sibling competition context. By showing their personality, bolder females may have a preferential access to different limited resources, like parental care. However, we cannot rule out that the expression of this trait may work during the post-fledgling dependence period. In this context, that is non-exclusive with the former, bolder females will gain a preferential access to other important resources like for instance, wintering territories an important feature associated to offspring survival.

INTRODUCTION

The individuals within a single population do not equally respond to the same stimuli, differing consistently in several aspects of their behaviour (Gosling 2001). This behavioural variability has been associated with individual survival and reproduction (Cote & Clobert 2007; Boon, Réale & Boutin 2007; Smith & Blumstein 2007; van Oers *et al.* 2008; Réale *et al.* 2009), suggesting that it can be modulated by natural selection. Different mechanisms have been proposed to explain this between-individual variation in behaviour. On the one hand, the behavioural variability has been explained by a divergent resolution of some life-history trade-offs (Sih, Bell & Johnson 2004; Wolf *et al.* 2007; Stamps 2007). For instance, bold individuals that have an increased reproductive success, may also incur in higher mortality rates when compared to shy individuals (Smith & Blumstein 2007). An alternative explanation has been that the behavioural variability is maintained by fluctuating selection pressures caused by the environmental variability and frequency-dependent selection (Wilson 1998; Sloan Wilson *et al.* 1994). This between-individual behavioural consistency is known as animal personality, that can be defined as the inter-individual differences in behaviour consistent across time and contexts (Sih *et al.* 2004; Réale *et al.* 2007; Stamps & Groothuis 2010; Griffin, Guillette & Healy 2015). Consistency does not imply that individual behaviour is fixed through different situations, but that the between-individual differences in behaviour do not change in different contexts (Biro & Stamps 2008; Stamps & Groothuis 2010). Personality can be explained through different axes, including: activity, shyness-boldness, exploration-avoidance, aggressiveness, and sociality (Réale *et al.* 2007). Shy-bold is one of the most studied behavioural axis (Huntingford 1976; Coleman & Sloan Wilson 1998; Carter, Goldizen & Tromp 2010; Fargallo *et al.* 2014) and is correlated to other personality traits like aggressiveness or exploration (Huntingford 1976; Coleman & Sloan Wilson 1998; Koolhaas *et al.* 1999; Biro & Stamps 2008). Along this axis, bold individuals are characterized to be more aggressive and showing more risky behaviours when compared to shy individuals, that are more reactive and have a lower propensity to take risks (Groothuis & Carere 2005). However, our knowledge about variation of personality during development is scarce (Fucikova *et al.* 2009). Taking into account that there is a strong positive selection over juvenile survival, exploring the variations of personality during this stage is crucial to fully understand the behavioural traits that best explain survival and dispersal during youth. In wild conditions, it is widely accepted that individual variation in behaviour of birds during the nestling stage or during the post-fledging dependent period can be associated with an enhanced expression of sexual (Vergara & Fargallo 2008) or social signals (Fargallo *et al.* 2014) in order to improve survival prospects. However, it is yet unexplored whether these signals can be linked to personality traits, particularly during early life.

Melanin-biased traits are good candidates to explore the association between signal expression and personality. This link is supported by the pleiotropic effects of the genes involved in the synthesis of melanin, since they can be responsible for the production of

melanin and may influence personality traits (Ducrest, Keller & Roulin 2008). Mechanistically, the association between the expression of melanin-based traits and behaviour has been explained due to the physiological effects of testosterone, favouring aggressive behaviours (Alonso-Alvarez & Velando 2001; Wingfield *et al.* 1990) and modulating melanin production (Wilson 1983; Fargallo *et al.* 2007; Evans, Goldsmith & Norris 2000). Within a competition context, the expression of enhanced melanin-based signals favours the discrimination of dominant from subordinate individuals (Senar 2006). Further, the association between melanin-based traits and personality has been explored in different species like the masked booby (*Sula dactylatra*; Fargallo *et al.* 2014), the tawny owl (*Strix aluco*; Da Silva *et al.* 2013) or the Eurasian siskin (*Spinus spinus*; Mateos-Gonzalez & Senar 2012). As a general trend, the studies exploring the relationship between melanin-based traits and personality found that darker individuals usually display bolder and more aggressive behaviours (Jawor & Breitwisch 2003; Mafli, Wakamatsu & Roulin 2011; van den Brink *et al.* 2012; Mateos-Gonzalez & Senar 2012 but see van den Brink *et al.* 2011; Fargallo *et al.* 2014). Still, the vast majority of these studies are biased towards adult individuals, and the information about this association in individuals during growth is scarce and the studies exploring it have provided contrasting results. For example, in barn owl (*Tyto alba*) nestlings a negative association between melanin-based colouration and docility has been described (van den Brink *et al.* 2011). However, the opposed results were found in a similar study preformed in common kestrels (van den Brink *et al.* 2012).

Juveniles of many species display colourations that differ in certain degree from those exhibited by adults (Moreno & Soler 2011). In the case of sexually dimorphic species juvenile colouration is usually a pale representation of females' plumage. Different ideas have been proposed to explain the role of juvenile colouration. It has been stated that it can work in a juvenile-adult communication context, by signalling juvenile subordination to adults in order to avoid agonistic encounters (Lyon & Montgomerie 1986). Alternatively, juvenile colouration may be signalling individual quality, modulating parental effort (Penteriani *et al.* 2007; Galván, Amo & Sanz 2008; Ligon & Hill 2010). In addition, juvenile plumage can also signal dominance and competitive ability within the juvenile age class (Jones 1990; Vergara & Fargallo 2008; Vergara, Fargallo & Martínez-Padilla 2010; Tringali & Bowman 2012). Finally, by expressing a cryptic plumage, juveniles may obtain benefits in terms of reduced predatory risks (Stutchbury 1991; Slagsvold, Dale & Kruszewicz 1995). Previous studies in birds show, that the expression of melanin-pigmented traits in juveniles can be a channel to communicate different behavioural characteristics. For example, the brown patch present in masked boobies is a proxy of juvenile personality, that can work as signal of competitive skills (Fargallo *et al.* 2014). This study stresses the importance of studying the role of juvenile colourations, as they can develop an important signalling role during this crucial life-stage. This importance is confirmed by a recent study showing an association between the expression of a melanin-based traits and juvenile survival, suggesting that the expression of juvenile colourations is associated with fitness (López-Rull *et al.* 2016).

Using common kestrels (*Falco tinnunculus*) as study species, we investigate the association of plumage blackness and rump colouration (two melanin-based traits) with personality in 148 nestlings during the breeding seasons of 2015 and 2016. We selected these melanin-based traits based on previous studies that highlighted their role during the first life-stages of kestrels. On the one hand, rump colouration, is a proxy of the competitive capabilities of the offspring during the post-fledgling dependence period (Vergara & Fargallo 2008) and fledging survival to adulthood (López-Rull *et al.* 2016). On the other hand, the length of the tail band (a black plumage trait), has been positively associated with nestling aggressiveness (van den Brink *et al.* 2012). We measured 5 personality traits and to two melanin-based traits, plumage blackness and rump colouration. According to previous studies showing a positive association between blackness and aggressiveness (van den Brink *et al.* 2012) we predict that blacker nestlings will display bolder personalities. In addition, taking into account that in this species individuals with greyer rumps have enhanced competitive abilities (Vergara & Fargallo 2008), we predict that individuals with greyer rumps will also have bolder personalities.

METHODS

Study species

The common kestrel is a medium sized diurnal raptor sexually dimorphic in size (females 20% bigger than males) and in plumage colouration (Village 1990). Nestlings start developing their plumage when they are 2 or 3 weeks old, and maintain it till the end of the first breeding attempt (Village 1990). Juvenile plumage is similar to adult females, brown on the head, back and upper-side of the wings, with black bars (López-Idiáquez *et al.* 2016). Nestling rump colouration presents a variable proportion of grey colouration in both males and females (mean 9.594%±1.428, range 0-70%, n=148). During approximately the first two weeks of the nestling period, females deliver the prey to the nestlings (Fargallo *et al.* 2003). After that, parents leave the preys inside the nest, and nestlings compete for it since they are able to dismember and eat the preys by themselves (Village 1990). In our population, nestling period has a mean duration of 32 days (Vergara & Fargallo 2008). During this period, females show higher competitive capacities than male nestlings when competing for the preys delivered by the parents (Fargallo *et al.* 2003). After that, during the post-fledgling dependence period, males are the more successful sex when competing for parental feedings (Vergara & Fargallo 2008).

General procedures and study area

The study was performed in the Campo Azálvaro region (40°40'N, 4°20'W), a treeless grassland, located in central Spain (1300 meters a.s.l.). In the area, 62 nest-boxes were put up progressively between 1994 and 2005, period during which the population was monitored in a long-term and individually-based fashion (Fargallo *et al.* 2001). During

the breeding seasons of 2015 and 2016, nests were visited every two days to detect laying date (day the first egg was laid), and to record clutch size (mean=4.85±0.12, range=3-7, n=49) and number of fledglings (mean=3.38±0.27, range=0-6, n=49). When nestlings were 25 days-old, we recorded their weight (to the nearest g), wing size (to the nearest mm), took a blood sample for molecular sexing (Fridolfsson & Ellegren 1999) and assessed plumage colouration. We took a digital photograph to assess plumage blackness (see below) and measured the proportion of grey colouration of the nestling rump (0% corresponded to a completely brown rump and 100% to a completely grey rump) following Vergara *et al.* (2009).

Blackness of nestling plumage

We analysed the photographs taken during the nestling handling using Adobe Photoshop CS6. All photographs included the back and right wing of all nestlings, were taken under the sunshade and included a scale to determine the size of the traits measured. Using the photographs taken during the capture, we measured 4 different traits: the tail terminal bands (TTB), the superior tail bands (STB), the rump bands (RB), and the wing blackness (WB). The first 3 measurements corresponded to the mean value of the width of the two central tail bands for the TTB. The values for STB and RB were calculated as the mean band width measured in three and four different feathers of the left side of the body respectively. WB was measured as the proportion of black colouration in a standardised area in size of the wing coverts. All measurements were highly repeatable r>0.82 (Supplementary Material-SM 6.1). In order to obtain a single variable representing a proxy plumage blackness of the nestlings, these variables of colouration were combined in a Principal Component Analysis (PCA; following López-Rull *et al.* 2016).

Behavioural assays

When nestlings were 25 days of age and before taking any of the biometrical measurements described above, we performed two different behavioural tests to assess their personality. The first one, to measure their escaping behaviour and the second one to explore individual aggressiveness. Tests were performed in each nestling, that were sequentially picked one by one from the nest. All tests were performed in an area close to the nest but avoiding the nest entrance to prevent being observed by the nestlings remaining in the nest.

Regarding the first set of tests, about escaping behaviour, we first quantified the distance to which fledglings run away in one minute by the number steps from the point where we left the nestling to where it moved away (variable 1: "*escape distance*"). After that time, the nestling was taken from the ground, and its response to handling was recorded and scaled from 0 to 4 (variable 2: "*handling response*"). The lowest value (0) corresponded to nestlings that remained still when being collected without any sign of aggressive response. The highest value (4) was assigned to individuals that reacted aggressively

when they were taken from the ground, laying on their backs and using their talons to defence themselves.

In relation to the second set of tests, about aggressiveness, we performed a restraint test to evaluate nestling aggressive behaviour. Specifically, aggressive behaviour was assessed by slowly offered and took away a gloved hand 3 times (5 second interval between each trial), as previously described in other species (Viñuela, Amat & Ferrer 1995; Fargallo *et al.* 2014). Briefly, we counted the number of times the nestling pecked and/or grabbed the gloved hand (variable 3: *"number of pecks"*) and the intensity of the response (variable 4: "*pecking intensity*"). The intensity of the response was scaled in 5 levels, from 0 (no response) to 4 (very aggressive response).

Finally, we determined nestling resistance to handling during the behavioural tests, in 5 levels (variable 5: "*handling resitance*"). These five levels ranged from 0 (quiet chicks that did not chirp or bite at any time) and 4 (chicks that hardly tried to escape biting and chirping continuously).

We estimated the repeatability of all behavioural traits performing the same tests the following day in a subset of 29 nestlings. All variables were repeatable, ranging from r=0.45 to r=0.88 (see SM 6.1 for further information about the repeatability of each variable). Our 5 behavioural traits were included in a PCA with the aim of obtaining a single variable as a proxy of nestling personality.

Statistical approach

In our analyses, we used Linear Models (LM) in R statistical software using the package "*lme4*" (Bates *et al.* 2015). As dependent variable, we included the mean-centred value, to year and nest, of the first axis of the PCA ($personality_c$ – see below), obtained by combining all behavioural traits in a PCA (see Below). As explanatory variables, we included plumage blackness, rump colouration, wing size, nestling sex and weight, and the interactions between sex and plumage blackness (see below), rump colouration, weight, and wing size. In order to check for the potential collinearity of our explanatory variables we calculated the Variance Inflation Factors (VIFs) using the package "*usdm*" (Babak 2015) without finding any evidence of collinearity (all VIFs<1.12, see SM 6.2 for further information). The combination between all variables were fitted and the more plausible models were selected using the Akaike Information Criteria for small samples (AIC_c), using the package "*MuMIn*" (Barton 2016). Models with smaller AIC_c values are preferred, and those models that differed in more than 2 units in relation to the smaller AIC_c were not considered plausible at explaining the variance of the dependent variable (Burnham & Anderson 2002).

RESULTS

Principal Component Analyses

First, regarding the variables of colouration, the PCA retained a single axis (PC1 - "*Plumage blackness*") that explained 45.8% of the variance (Table 6.1). This axis was positively associated with the included variables, representing higher values of the axis blacker individuals. Second, the PCA of the personality variables retained only the first axis (PC1 - "*Personality*") that explained 52.8% (Table 6.2) of the variance. All behavioural traits included in the PCA of "*Personality"* were negatively correlated, except run away distance that had a positive association. Thus, this axis represents a shy-bold gradient, in which higher values are associated with shyer behaviours. Since this "negative" association between the values of the axis and the behaviour may seem counterintuitive, we multiplied it by -1 for a more intuitive interpretation, in which positive values of the axis correspond to bolder individuals.

Component	Percentage of explained variance	Cumulative percentage of explained variance
PC1	45.84	45.84
PC2	25.53	71.37
PC3	16.50	87.87
PC4	12.13	100

Table 6.1: Result of the Principal Component Analysis (PCA) summarizing our measurements of nestling plumage blackness.

Component	Percentage of explained variance	Cumulative percentage of explained variance
PC1	52.81	52.81
PC2	20.32	73.13
PC3	17.15	90.28
PC4	7.95	98.23
PC5	1.77	100

Table 6.2: Result of the Principal Component Analysis (PCA) summarizing nestling behavioural variables obtained in the personality tests.

Variables	AIC_c	ΔAIC_c
Blackness*Sex	450.947	0
Blackness*Sex + Wing size*Sex	451.040	0.093
Blackness*Sex + Weight	452.980	2.033
Blackness*Sex + Rump coloration	452.099	2.046
Blackness*Sex + Wing size	453.095	2.148
Blackness*Sex + Wing size*Sex + Rump coloration	453.109	2.161
Blackness*Sex + Wing size*Sex + Weight	453.200	2.253
Rump Colouration*Sex + Blackness*Sex + Wing size*Sex	453.895	2.948
Rump Colouration*Sex + Blackness*Sex	453.981	3.033
Weight*Sex + Blackness*Sex	454.379	3.432

Table 6.3: Results of the model selection procedure for the association between personality, colouration and size. Models are ranked according to AIC_c values. In the models including an interaction (*), independent terms were also included.

Our results show that there are two equally plausible models according to the AIC_c values. The best model included the interaction between plumage blackness (Table 6.3 and 6.4). The second-best model included the interactions of sex with plumage blackness and wing size (Table 6.3 and 6.5). We further explored these models and split both interactions in order to explore the slope and directionality of the sex-dependent associations between personality and blackness or wing size (Figure 6.1a and 6.1b respectively). We found a statistically significant positive relationship between personality and plumage blackness in females but not in males (Table 6.6, Figure 6.1b). We did not find any statistically significant relationship between personality and wing size for either males or females.

Trait	Estimate	*SE*	*F*	*P*
Blackness	**0.270**	**0.103**	**$F_{1,144}$=2.607**	**0.009**
Sex	-0.196	0.202	$F_{1,144}$=0.717	0.398
Blackness*Sex	**-0.403**	**0.149**	**$F_{1,144}$=7.320**	**0.007**

Table 6.4: Results of the linear model exploring the association of personality (*$Personality_c$*) with plumage blackness, and sex in common kestrel nestlings. In order to account for the variability within years and nest *$Personality_c$* was mean-centred to those variables. Significant factors are shown in bold.

Trait	Estimate	*SE*	*F*	*P*
Blackness	**0.259**	**0.102**	**$F_{1,142}$=2.646**	**0.012**
Wing Size	0.019	0.014	$F_{1,142}$=0.242	0.176
Sex	**7.821**	**3.940**	**$F_{1,142}$=0.608**	**0.049**
Blackness*Sex	**-0.403**	**0.148**	**$F_{1,142}$=7.333**	**0.007**
Wing Size*Sex	**-0.048**	**0.023**	**$F_{1,142}$=4.145**	**0.043**

Table 6.5: Results of the linear model exploring the association of personality (*$Personality_c$*) with plumage blackness, wing size and sex in common kestrel nestlings. In order to account for the variability within years and nest *$Personality_c$* was mean-centred to those variables. Significant factors are shown in bold.

Trait	Estimate	*SE*	*F*	*P*
		Females		
Blackness	**0.270**	**0.107**	**$F_{1,76}$=6.284**	**0.014**
Wing Size	0.019	0.015	$F_{1,75}$=1.677	0.199
		Males		
Blackness	-0.143	0.100	$F_{1,67}$=1.747	0.159
Wing Size	-0.027	0.017	$F_{1,68}$=2.303	0.134

Table 6.6: Results of the Linear Models exploring the association of personality with plumage blackness, and wing size in both males and female nestlings. In order to account for the potential influence of year and nest, we mean-centred the values of personality to these factors. Significant variables are in bold; values for the excluded and non-significant variables refer to the step before their exclusion.

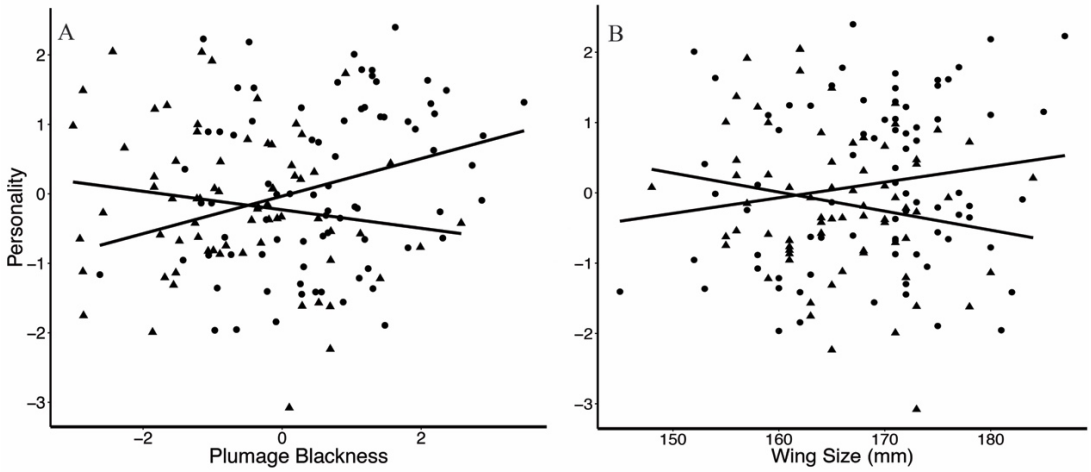

Figure 6.1: Relationship between personality and plumage blackness (A), and between personality and wing size (B) in common kestrel nestlings. Dots represent females and triangles represent male nestlings.

DISCUSSION

Our results suggest that the association between melanin-pigmented traits and personality in kestrel nestlings is sex-dependent. We found a positive association between plumage blackness and boldness in female but no male nestlings. Further, the association between melanin-pigmented traits and personality is only significant for this specific melanin-coloured trait. The lack of correlation between greyness of the rump and personality suggests that the association between personality and the expression of melanic colouration in nestlings is trait-dependent.

Our results show that the association between personality and plumage blackness is mediated by the sex of the nestling. Females show a positive association between blackness and personality, being blacker females those that display bolder behaviours. We consider that the positive association between bolder females are darker colouration can be explained under two signalling contexts, in both cases related to the higher energetic needs that females (the bigger sex) had during development. Firstly, under a parent-offspring scenario, different studies have shown that the colourations exhibited by juvenile individuals can modulate the amount of care they receive from their parents (Penteriani *et al.* 2007; Galván *et al.* 2008). For example, in eagle owls (*Bubo bubo*) the brightness of the white feathers surrounding owlets mouth modulates the amount of food provided by the parents (Penteriani *et al.* 2007). This is expected because parents increase their fitness by biasing the amount of care they provide towards the individuals with higher expected fitness return (Stamps 1990). In kestrels, since female nestlings are the bigger sex and has the highest energetic needs, showing a darker colouration can increase the chances to increase parental care to ensure their energetic demands. Alternatively, plumage blackness may be working as a signal within the juvenile age-class instead of being a signal to the parents. Previous studies have shown that juvenile colouration can be a signal of dominance and competitive ability (Jones 1990; Vergara & Fargallo 2008). By expressing darker phenotypes, dominant individuals may gain priority access to limited resources, reducing the costs associated with dominance (Senar 2006). The expression of blacker plumages may confer different kind of benefits to the juvenile females. By one hand, this dominance signal can allow blacker females to have an increased access to the resources provided by the parents during the post-fledgling dependence period. During the post-fledging period, parental feeding is dominated by male offspring, which signal their competitiveness abilities showing a higher proportion of grey in their rumps (Vergara & Fargallo 2008). We think that females, in spite of not being as competitive as males during the post-fledging period, may use their colouration as competitive signal among females. On the other hand, the benefits may extend far beyond the dependence period, and females exhibiting darker plumages, may have a better access to high quality wintering territories. This enhanced access to the best territories may occur because bolder individuals usually display more aggressive behaviours (Sih *et al.* 2004; Rudin & Briffa 2012), that is usually an index of success in contest competition. These two ideas are not mutually exclusive and plumage blackness may be working in both contexts, as a signal to the parents and as a signal to the other juvenile individuals. Independently of the signalling contexts our results suggest that female kestrels obtain benefits by expressing blacker plumages. This is in agreement with a recent study in kestrels showing that female juveniles with blacker plumages have increased recruitment rates (López-Rull *et al.* 2016). Thus, suggesting that females benefit from being bolder during the first stages of their life.

Interestingly, our results show that rump colouration is not associated with our shy-bold behavioural axis. As stated above, rump colouration is an index of male competitive ability of offspring during the post-fledging dependence period (Vergara & Fargallo 2008). Fledglings with greyer rumps are able to capture a greater number of the preys

provided by the parents than fledglings with browner rumps (Vergara & Fargallo 2008). This lack of association can be explained by different personality axis to the one studied here. For example, competitive ability has been associated with the proactive-reactive (David, Auclair & Cézilly 2011), rather than to the shy-bold axis. In addition, we cannot rule out that the melanin-pigmented traits studied here and rump colouration can play a different role between males and females.

Broadly, our study stress the importance of juvenile plumages during early life, including the nestling period. According to our results, we defend that exhibiting specific melanin-pigmented traits, but not greyness in the rump, juvenile females may signal their dominance and obtain different parental benefits during the first year of life.

ACKNOWLEDGEMENTS

We thank the Finat family for allowing us to work on their property. Juan San Teodoro provided logistical support, and Alba Estrada helped with the calculations of the VIFs. This is a contribution of El Ventorrillo Biological Station. The study was financed by the Spanish Ministerio de Economía y Competitividad (Project: CGL2013-42451-P)

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SUPPLEMENTARY MATERIAL

SM-6.1 Repeatability values

Repeatability values (Lessells & Boag 1987) for the variables included in the Principal Component Analyses (PCA) of the colouration measurements (Table 1) and of the behavioural test (Table 2).

Trait	r	*P*	N
Wing Blackness	0.829	<0.001	20
Tail Terminal Bands	0.968	<0.001	20
Superior Tail Bands	0.971	<0.001	20
Rump Bands	0.885	<0.001	20

Table SM 6.1: Repeatability values for the colouration measurements.

Trait	r	*P*	N
Handling resistance	0.883	<0.001	29
Escape distance	0.453	0.005	29
Handling response	0.544	0.0008	29
Number of pecks	0.790	<0.001	29
Pecking intensity	0.680	<0.001	29

Table SM 6.2: Repeatability values for the personality measurements.

SM-6.2 Variance Inflation Values

Variance Inflation Factors (VIFs) for the explanatory variables included in the model explaining the differences in personality between the different nestlings (Table 3). VIFs were calculated in R statistical software using the package "*usdm*" (Babak 2015).

Trait	VIF
Plumage blackness	1.063
Weight	1.120
Wing size	1.021
Rump colouration	1.117

Table SM 6.3: Variance Inflation Values (VIF) of the explanatory variables included in the model exploring the variability in nestling personality.

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Chapter 7

DISCUSSION

Disentangling the factors that determine the covariation of signal expression and behavioural response is needed to comprehend the evolution and maintenance of stable communication systems. In this PhD, I have explored the mediating effect of environmental and intrinsic factors on the functionality, fitness costs and behavioural responses of the expression of different melanin-based traits, using common kestrels (*Falco tinnunculus*) as study species. First, in Chapters I and II, I have explored the independent or interactive effects of age and environment in the expression of two melanin based traits, the spottiness of the mantle and rump colouration in breeding kestrels. Second, Chapters III, IV and V are focused on the role that melanin-based traits play within a behavioural perspective. Specifically, I explored the behavioural response associated with their expression, both in signallers and receivers. Chapter III, experimentally tests the role of female rump colouration within a female-female competition context. Chapter IV, explores the role of fledglings' rump colouration during the PFDP and Chapter V, deals with the role of plumage blackness and rump colouration as signals of offspring's personality.

Signal expression can be modulated by different factors, some associated with the characteristics of the individual, like age or sex, and other that are dependent of the environments where they live. Many studies have shown that the expression of certain signalling traits changes with age, however the vast majority have a cross-sectional rather than a within-individual perspective (Velando, Drummond & Torres 2010; Forstmeier *et al.* 2006). Separating the within- from the between-individual effects of age is crucial in order to being able to detect the individual-level trends, that can be hidden by demographic heterogeneity (Vaupel & Yashin 1985; van de Pol & Wrigth 2009). The results in Chapters I and II, reveal that the number of back spots in male kestrels and rump colouration in females show a within-individual age-dependence. Broadly, these results suggest that age is an important factor modulating the expression of melanin-based traits. From an evolutionary perspective, they can be explained by the change in the resource allocation priorities predicted by life-history theory, where individuals prioritize investment in survival in early age and in ornamentation at late life (Stearns 1992). Thus, we defend that the framework that best explains the age-dependent variation of the expression of the melanin-pigmented traits is life-history theory, rather than senescence-based explanations. From a behavioural point of view, the patterns found for the number of spots and female rump colouration can also be explained by female choice and increased experience in agonistic encounters with age, respectively. First, female mating

with the less spotted males at late-age, may benefit of having more experienced males. This is justified by our results in Chapter I, where I found that old males have larger clutches and raise more offspring. Second, the pattern found for rump colouration can be explained by an increased competitive ability with age, associated with the experience acquisition through the participation in successive encounters (Arcese & Smith 1985). These enhanced competitive abilities lead to increased resource availability, that will allow females to invest more resources in signalling (Arcese & Smith 1985; Stahl *et al.* 2001; Verhulst *et al.* 2014). I also explored the effects of food availability, as a proxy of environmental conditions, on the above-mentioned traits. The role of the environmental conditions on the expression of melanin-based traits has been subject of intense debate, with some studies claiming an independence from the environmental conditions (Roulin & Dijkstra 2003; Niecke, Rothlaender & Roulin 2003) and others stating that these traits are sensitive to them (Fargallo *et al.* 2007; Vergara *et al.* 2009). Nowadays, the role of environmental conditions on the expression of melanin-based traits is unquestionable, and the results found in this PhD partially support this idea since I found that the size of the spots is positively linked to food availability. However, I did not find any significant result in the association between food availability and rump colouration. This is surprising, since a previous study suggested that the additive genetic variation of the expression of the rump is low, particularly when compared with residual variance which can be understood as a proxy of environmental heterogeneity (Kim *et al.* 2013). I only studied the effects of one environmental factor, food availability, and the possibility that rump colouration is modulated by other environmental factors cannot be ruled out. In Chapter I we also found that environmental conditions not only modulate the expression of a trait, but also the selection pressures behind trait expression, supported by a negative association between the number of spots and clutch size for old individuals under harsh environmental conditions. This result evidences that environmental heterogeneity not only modulates trait expression but may also strengthen the selection on the expression of the traits. In Chapters I and IV, it is described that the factors driving the expression of the traits, and their behavioural significance are sex dependent. In Chapter I, the correlations found between the number and size of spots, and age and food availability, respectively were only significant in males, but not in females. I defend that, this divergent pattern arises due to the different role that these traits play in males and females, and thus on the selective pressures that modulate their expression. My results point out that, in males, mantle spottiness may work under a sexual context being a signal of individual quality. In females, however, this trait is not sexually selected and it expression may be driven by other selective pressures. In Chapter V, the results show that the association between personality and plumage is significant only in females but not in males. I believe that this different integration of personality and blackness between males and females may have physiological roots, probably associated with testosterone due the prominent role of this hormone modulating both melanogenesis (Jawor & Breitwisch 2003) and behaviour (Alonso-Alvarez & Velando 2001). However, further research is needed to shed light on the sex-dependent mechanisms that drive the different functionality and behavioural responses of melanin-pigmented traits.

The main role of melanin-based traits takes place within a social context, signalling dominance (Senar 2006). Several studies have proven this role by showing how an enhanced expression of these melanin-based badges leads to a discrimination of dominant from subordinate individuals (Rohwer 1975; Senar *et al.* 1993). Still, the vast majority of the studies are biased towards males, leaving the role of melanin-based signals in females underexplored. In Chapter III, I experimentally test whether female rump colouration works as a badge of status within the communication system. Specifically, my results evidence that displaying greyer rumps is associated with a higher position in the social hierarchy of females in our population. Interestingly, this result, in addition to the above-mentioned results of Chapter II, suggests that female dominance increases with age, as females increase the proportion of grey colouration in the rump as they age. A increasing pattern of greyness of the rump with the age of female is consistent with a previous study where a within-individual increase in dominance was found (Verhulst *et al.* 2014). However, the expression of melanin-pigmented traits in kestrels is not only shown during adulthood, but during the early stages of life. In many species, juveniles exhibit colourations markedly different from those exhibited during adulthood (Moreno & Soler 2011). In spite that the main role of juvenile colouration has been associated to signalling subordination to adults (Senar 2006), different studies suggest that it can also work signalling quality to the parents (Penteriani *et al.* 2007; Lyon, Eadie & Hamilton 1994), and competitive abilities to other individuals within the same age-class (Jones 1990; Vergara & Fargallo 2008).

I explored the potential association between the expression of melanin-pigmented traits and personality in order to elucidate the role of these signals as indexes of competitive abilities within individuals of the same age. The results of Chapter V, reveal a positive correlation between female plumage blackness and boldness. I suggest that this association can be framed within a social context and that by exhibiting enhanced versions of this trait, females may increase their chances to get access to limited resources particularly during the post-fledging period, avoiding the costs associated to agonistic interactions (Rosvall 2011). A recent study support this idea, since female nestlings bearing blacker plumages increased their survival prospects during winter (López-Rull *et al.* 2016). However, the signalling role of melanin-pigmented traits among siblings does not have to be constrained within the nestling stage, and it can also work during the post-fledging period. I explored how the expression of melanin-pigmented traits was associated with the length of the post-fledgling dependent period in order to infer a potential signalling context, in addition to its potential fitness consequences. Specifically, I studied the association between rump colouration and the duration of the PDFP with survival to the first winter. I tackled this question because previous studies have shown that rump colouration plays an important role, with fledglings exhibiting greyer rumps being more competitive (Vergara & Fargallo 2008). In spite of this, I did not find a significant association between rump colouration and survival, that was driven by the duration of the PDFP. I think that this result can be explained through the benefits that fledglings gain in terms of parental care. Parental feeding during this period is probably one of the main factors determining offspring mass at independence, a key trait

determining first-winter survival (Bouwhuis *et al.* 2015). Interestingly however, we found that males that provided longer post-fledgling dependence periods had reduced clutches during the following reproductive season, suggesting that the care provided during the post-fledgling is a costly activity.

Broadly, the results described in this PhD highlights the role of melanin-based traits in different communication context across different life-stages in common kestrels, where they carry out different signalling functions. In adults, the spottiness of the mantle in males may works as a secondary sexual trait signalling individual quality to females. I also found that female rump colouration is a badge of status, signalling dominance in a female-female competition context. In addition, my results also suggest that females may increase their dominance as they age, as the expression of rump colouration presented a within-individual increase. When it comes to the offspring, I found that plumage blackness may act as a proxy of female boldness modulating the access to limited resources during the first-stages of life. Finally, I was not able to find any association between offspring rump colouration and recruitment rates, that were driven by the duration of the post-fledgling dependence period. Overall, the results obtained in this PhD support the idea that melanin-pigmented traits are modulated by both individual and environmental factors and their expression shape the behaviour response of receivers. I suggest that for a fully behavioural and evolutionary comprehension of the role of these traits in animal communication systems, it is needed a further deepening on the role of environmental variation on trait expression and on the association between the expression of these traits and fitness.

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Chapter 8

CONCLUSIONES

1. La edad de los individuos es un factor que puede modular la expresión del número de motas del manto de los machos y de la coloración del obispillo de las hembras, ambos basados en melanina. De acuerdo con la teoría de historias de vida, esta dependencia de la edad podría explicarse por una mayor inversión energética en ornamentación a medida que se reduce el valor reproductivo residual de los individuos.

2. Nuestros resultados muestran que la expresión de varios de los caracteres melánicos estudiados es dependiente del sexo. Este resultado sugiere que los rasgos melánicos podrían estar integrados de forma diferente en machos y hembras, probablemente debido a las diferentes presiones selectivas inherentes a cada sexo. Por una parte, los rasgos expresados en los machos estarían más influenciados por la selección sexual y la selección de las hembras. Por la otra, los rasgos exhibidos por las hembras estarían modulados por la selección social y la competencia por recursos limitados.

3. La disminución en el número de motas con la edad podría funcionar como una señal de calidad de los machos. Las hembras obtendrían beneficios indirectos al emparejarse con machos más viejos, en forma de descendientes con genotipos "longevos". Además, las hembras emparejadas con machos más viejos también incrementarían su eficacia biológica ya que los machos aumentan su éxito reproductor a medida que envejecen.

4. La coloración del obispillo de las hembras adultas de cernícalo, específicamente la proporción de gris presente en este rasgo, funciona como una señal de estatus indicando la capacidad competitiva de las hembras. Estas señales crean una jerarquía que regula el acceso a recursos limitados reduciendo los costes asociados a los comportamientos agresivos. Nuestros resultados indican que las hembras reproductoras pagan el coste de una mayor agresividad con una reducción en el tamaño de puesta ya que encontramos una relación negativa entre el número de huevos y los niveles agresividad.

5. La coloración del obispillo de las hembras es dependiente de la edad, existiendo un incremento en la proporción de gris a medida que envejecen. No obstante, la expresión de la proporción de gris del obispillo de las hembras en el momento de la reproducción se mantiene a partir del tercer año de edad. Estos resultados sugieren que la dominancia que presentan las hembras de cernícalo también podría ser dependiente de la edad.

6. La correlación positiva entre la abundancia de alimento y el tamaño de las motas del manto de los machos evidencia que la expresión de estos rasgos melánicos es sensible a las fluctuaciones en la disponibilidad de alimento. Además, la heterogeneidad ambiental no sólo modula la expresión de la señal, sino que también puede influir en la asociación entre la expresión del rasgo y su eficacia biológica asociada. Sin embargo, la abundancia de alimento no se correlaciona con la expresión del gris del obispillo de las hembras, evidenciando que la sensibilidad de los rasgos melánicos a este factor ambiental es parcialmente dependiente del sexo y del rasgo estudiado.

7. La relación positiva existente entre la osadía de los pollos y la cantidad de negro en el plumaje evidencia que los rasgos basados en melanina también podrían tener un rol señalizador en las primeras fases de vida. Esta asociación podría funcionar en la competencia entre hermanos durante su estancia en el nido o entre volantones una vez que abandonan el nido, cuando compiten por recursos. Otra explicación no excluyente sería que fuera una señal para los padres, con objeto de obtener un trato preferencial durante la cría. Independientemente del contexto señalizador donde funcione la coloración estudiada, los resultados obtenidos indican la relación entre la personalidad y el grado de expresión de rasgos melánicos durante el crecimiento.

8. La duración del periodo de dependencia está positivamente asociada a la probabilidad de reclutamiento de los volantones, probablemente por los cuidados parentales recibidos durante ese tiempo. Los resultados sugieren además que estos cuidados provistos por los padres les inducen un coste reproductivo en la siguiente temporada de cría. A pesar la relación positiva existente entre la cantidad de gris en el obispillo y la duración del periodo de dependencia, nuestros resultados no muestran que el gris del obispillo influya en la probabilidad de reclutamiento. Esta falta de asociación puede deberse a que durante el invierno los pollos con plumajes más similares a las hembras (obispillos más marrones) son favorecidos por la selección. Por ello, a pesar de tener periodos de dependencia más largos, éstos resultan en mayores tasas reclutamiento.

AGRADECIMIENTOS

«La gratitud, como ciertas flores, no se da en altura y mejor reverdece en la tierra buena de los humildes»
-José Martí

Mucho ha de agradecer una persona en mi situación de "*desarrapado*" científico, aun así, voy a intentar que esto sea conciso y sucinto. Para comenzar, me gustaría agradecer el apoyo de mis padres durante la elaboración de esta tesis. Durante los tres primeros años de la misma he parasitado sus recursos, tanto económicos como logísticos, sin los cuales la tesis no hubiera podido ser realizada. No puedo olvidar, además, que fue Pablo Vergara quién tras acogerme bajo su ala durante la elaboración de la tesis de maestría, me mostró el comienzo del camino a recorrer en este mundo que es la ciencia y por ello le estaré eternamente agradecido. Jesús T. García y Paco García González me dieron la oportunidad de trabajar en el Instituto de Investigación en Recursos Cinegéticos (IREC) y en la Estación Biológica de Doñana (EBD) respectivamente, a donde fui siguiendo el peregrinaje laboral de Jesús Martínez-Padilla. Gracias a ellos, tuve la inestimable oportunidad de conocer a científicos y personas increíbles, de los que me llevo un gran recuerdo, tanto en Ciudad Real como en Sevilla. No puedo evitar agradecer a mis compañeros de despacho(s) su apoyo y ayuda durante estos años: Jesús, Miguel, Eduardo, José Manuel "J", Paloma, Sara I y Sara II, muchas gracias. Juan Antonio Fargallo merece una mención especial, no solo por financiar nuestros estudios, sino también porque gracias a él he podido trabajar en una institución como el Museo de Ciencias Naturales (MNCN), donde también he coincidido con gente maravillosa (Alex, Octavio, Jimena, Gonzalo, Elisa, Roger, Eva, Indra, y muchos más). Trabajar en el Museo me ha permitido visitarlo en innumerables ocasiones durante mis jornadas de trabajo (no sé si debiera de confesar esto) en busca de inspiración y sosiego. Además, gracias a Juanto he podido disfrutar de 5 insuperables veranos viviendo en el Ventorrillo y dedicándome a coger cernícalos en Campo Azálvaro, eso sí, sin ver un mísero lobo (la observación más codiciada por esos lares). Juan Navarro ha sido un infatigable compañero de aventuras tanto en el Ventorrillo como el campo y sin él y su irreprochable actitud, ánimo y buen humor, esto no hubiera sido lo mismo. Me tengo que expresar en los mismos términos hacia Isabel López-Rull, con quien he compartido largas jornadas de campo estos últimos dos años. ¡Muchas gracias a los dos! Tampoco puedo olvidar mis aventuras por los campos de Castilla con Alfonso y Carlos, pasando al principio, frío y después mucho calor, como escribió Delibes en Las Ratas: "*En Castilla, ya se sabe, 9 meses de invierno y 3 de infierno*". Durante estos años no todo ha sido ciencia, afortunadamente, quisiera por ello destacar y agradecer el papel que jugaron los inquilinos, presentes y pasados, de La Comuna en mi formación humanística durante estos 4 años. Finalmente, y pese a que no tengo palabras para describir cuan agradecido le estoy, tengo que destacar el papel de Jesús Martínez-Padilla al frente esta tesis. Durante la misma, ha sido el perfecto auriga que ha llevado a mi voluble espíritu y testarudo ser, de la forma más recta posible, a concluir esta odisea de forma más o menos exitosa.